Prenatal exposure to non-persistent chemicals and child neurodevelopment:
an epidemiological study

Michiel Arjen van den Dries

Prenatal Exposure to Non-persistent Chemicals and Child neurodevelopment: an Epidemiological Study

Prenatale blootstelling aan niet-persistente chemicaliën en neurologische ontwikkeling van het kind: een epidemiologische studie

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organophosphate pesticides and brain morphology and white matter microstructure in preadolescents. *Environmental research*, 191, 110047. (Chapter 8)

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Part I

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Chapter 1

General introduction

Introduction

Rationale

Exposure to chemical pollutants is a great and rising worldwide problem. The human health consequences of chemical pollution are poorly understood and almost inevitability underestimated.[1,2] Over 100.000 new chemicals have been introduced since 1950, and of those approximately 5000 are produced on an extensive scale.[2,3] This comprehensive production has led to a broad diffusion of chemicals in the environment and resulted in a universal human exposure from fetal life onwards. Further, only in the past decades pre-market safety assessments have been required in few developed countries.[1,2] The consequence is that chemicals whose safety and toxicity have never been thoroughly tested have repetitively affected human health and the environment in the past century.[1,2] Lead, dichlorodiphenyltrichloroethane (DDT), and asbestos are such examples. In the past decades, novel chemicals with little pre-market safety assessment have been introduced on a global scale resulting in a ubiquitous exposure.[1,2,4] Three of these chemical groups are phthalates, bisphenols and organophosphate (OP) pesticides and are commonly found in variety of consumer products and food items.[5,6]

Phthalates are chemicals exert as plasticizers and as solvents in numerous consumer goods.[7] The annual worldwide production of phthalates is estimated to be 5 billion kg.[8] There are several different sorts of phthalates with diverse usages and chemical characteristics. For example, Di-(2-ethylhexyl) and benzyl butyl phthalates are typically used as plasticizers for polyvinyl chloride (PVC) and exist in products such as food packaging materials, floor materials, clothing, toys, and medical devices. Whereas diethyl and dibutyl phthalates are generally used as solvent and fixative in products such as cosmetics, paints, and glues.[7,9-11] Because phthalates are weakly bound to their product, they easily leach into the environment and therefore are present in a variety of consumer goods and food items. Phthalates are also commonly detected in indoor air and house dust.[7,12-15] Consequently, the exposure to phthalates most likely happens through inhalation of air from the indoor environment, diet, and the use of consumer goods such as personal care products, and products containing PVC.

Bisphenol A and its replacements (such as bisphenol F and bisphenol S) are among the most widely used group of synthetic compounds around the globe. These chemicals are mainly applied in the production of polycarbonate plastics and epoxy resins and therefore present in water bottles, storage containers, and in the lining of food and beverage containers. Further, bisphenols are present on thermal paper receipts.[16,17] Similar to phthalates, bisphenols are weakly bound and leach from their product into its contents.[18-21] Therefore, diet is the main source of bisphenol exposure.

Pesticides are extensively used in both developed and developing countries as a result of an intensification in agriculture largely for export purposes.[22] For instance, the Netherlands is one of the world's leading exporter of agricultural products with an estimated export value of 90.3 billion euros in 2018.[23] As a consequence, more than half of the total surface area of the Netherlands is being utilized for agricultural purposes and more pesticides and fertilizers per square km of farmland are being applied as compared to most other western countries.[24,25] At present, 2.3 billion kg of pesticides are used worldwide of which one third consist of OP pesticides.[26] OP pesticides are insecticides and particularly used in agriculture for crop protection. After harvesting, residues of OP pesticides may remain on or in the agricultural product.[27] Therefore, the exposure to OP pesticides in urban settings mainly occurs through the consumption of food.[28]

Biomonitoring studies have shown that concentrations of phthalates, bisphenols, and OP pesticides are commonly detected in biospecimens of the general population.[29] These chemicals are non-persistent and therefore after ingestion, absorption, or inhalation are rapidly metabolized and excreted.[22,30,31] Concern exists about the long-term health effects of these non-persistent chemicals because the exposure through consumer products is ubiquitous, across the lifespan, and occurs in many different combinations and concentrations. Because these chemicals are coming from industry and are non-persistent, their contribution to adverse health effects is potentially preventable.

Prenatal exposure to these chemicals can occur because they have the ability to surpass the placenta and blood brain barrier.[32-38] The brain is particularly susceptible to neurotoxicity during fetal life. To ensure normal brain development, many vital biological actions occur during the fetal period.[39] Therefore, interference by chemical insults during these precisely timed processes may result in adverse neurodevelopmental health outcomes.[39-41] Animal studies have shown that low-dose exposure to phthalates, bisphenols, and OP pesticides impairs neurodevelopment and behaviour.[42-56]

In humans, few epidemiological studies have examined the association of fetal exposure and neurodevelopment, including cognition and behavioral outcomes such as autism and attention-deficit hyperactivity disorder. The results of these studies have been suggestive, but overall are inconclusive.[40,57-60] For example, one study found prenatal OP pesticide exposure measured at the 13 weeks and 26 weeks of gestation to be inversely associated with IQ,[61] whereas two other studies found no association between early, mid, and late pregnancy exposure.[62,63] Further, much uncertainty exists about which mechanisms underlie the observed associations between non-persistent chemicals and neurodevelopment.

The heterogeneity in epidemiological results may be explained by the fact that most of these studies had modest sample sizes. This may have reduced the statistical power to

consistently observe poor health outcomes. Further, most studies measured prenatal exposure to these chemicals at one, or at most two, time points during pregnancy. However, several potentially susceptible periods of fetal neurodevelopment to chemical exposure may exist during pregnancy.[41,64,65] A susceptible period is a specific developmental moment during which chemical exposure results in a greater effect on health than the exposure to the same chemical at another moment.[66] It is conceivable that some studies using a single exposure measurement during pregnancy might have missed the susceptible period, potentially resulting in diluted effects (i.e., estimates closer to the null). Moreover, the short half-life, i.e. fast metabolization and excretion of non-persistent chemicals, results in within-person variability in biomarker concentrations as a result of variation in contact with exposure sources (e.g., changing dietary patterns or use of different type of personal care products). Therefore, the use of a biomarker measurement at a single time point to assess pregnancy exposure to non-persistent chemicals may have resulted in exposure misclassification, also resulting in a regression of the exposure-response estimates towards the null.[60,67] Finally, the exposures to these non-persistent chemicals do not occur in isolation but coincide (as a mixture) on a daily basis across the lifespan. Most epidemiological studies on these chemicals in relation to neurodevelopment have been restricted to the investigation of associations between single pollutants and neurodevelopmental outcomes. Restricting analyses to single pollutants may ignore health effects which would be detected if the joint chemical exposure is assessed. For example, the additive effect of the exposure to multiple chemicals acting on the same biological pathways may be harmful even when individual exposures are below practically meaningful thresholds. Further, co-exposures may act together in different ways to produce unexpected synergistic health effects.[68-71] Other limitations of single-chemical models are the potential biased effect estimates in the presence of co-pollutant confounding, and inflated false discoveries when correlated exposures are modelled separately.[60,72] Concentrating on the mixture as a whole can provide effect estimates that more closely correspond to real-world exposures. Taken together, much uncertainty still exists about the relationship between prenatal non-persistent chemical exposure and neurodevelopment.

Aims

The present thesis examined the relationship between prenatal exposure to phthalates, bisphenols, and OP pesticides and neurodevelopment in children by studying (i) the determinants of exposure to non-persistent chemicals during pregnancy, (ii) exploring the association of prenatal exposure to non-persistent chemicals with neurodevelopment in children, and (iii) investigating the effect of exposure to these non-persistent chemicals on the potential mediators—such as thyroid function, brain structure, and fetal growth—of the association with neurodevelopment.

Setting

These aims were explored using data from the Generation R Study, a prospective population-based cohort designed to detect early environmental and genetic determinants of development from fetal life onward in a multi-ethnic urban population.[73] The Generation R Study is characterized by a large sample size, detailed follow-up information of prenatal and postnatal development of the fetus or child, repeated measurements (early, mid, and late pregnancy) of non-persistent chemical exposure, and the availability of detailed demographic information. Every women who was pregnant and lived in the research district Rotterdam, the Netherlands, and was expected to give birth between 2002 and 2006 were eligible.[73]

In total, 8,879 women were enrolled during pregnancy. Between 2004 and 2006, women were asked to provide biospecimens at the time of routine ultrasound examinations during early, mid, and late pregnancy. A total of 2,083 women provided a urine sample at each visit. When children turned 6 and 9 years of age, mother-child pairs were asked to visit the research center in order to collect sociodemographic data and biospecimens, and to measure health outcomes including neurodevelopment. Of the 2,083 women who provided a urine sample at each prenatal visit, 1,405 mother-child pairs provided data at the follow-up visits. The availability of follow-up data was a requirement to allow studies of the associations between prenatal non-persistent chemical exposure and child health including neurodevelopment. This subset is the basis of the majority of the studies presented in this thesis. Mothers provided written informed consent for themselves and their children. The Medical Ethical Committee of the University Medical Center Rotterdam approved the study.

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Part II

Determinants of exposure to non-persistent chemicals during pregnancy

2

Chapter 2

Determinants of organophosphate pesticide exposure in pregnant women: A population-based cohort study in the Netherlands

van den Dries, M. A., Pronk, A., Guxens, M., Spaan, S., Voortman, T., Jaddoe, V.W.V., Jusko, T. A., Longnecker, M. P., & Tiemeier, H. (2018).

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Abstract

Background: In the Netherlands organophosphate (OP) pesticides are frequently used for pest control in agricultural settings. Despite concerns about the potential health impacts of low-level OP pesticide exposure, particularly in vulnerable populations, the primary sources of exposure remain unclear. The present study was designed to investigate the levels of OP metabolite concentrations across pregnancy and to examine various determinants of OP metabolite concentrations among an urban population of women in the Netherlands.

Method: Urinary concentrations of six dialkyl phosphate (DAP) metabolites, the main urinary metabolites of OP pesticides, were determined at <18, 18–25, and >25 weeks of pregnancy in 784 pregnant women participating in the Generation R Study (between 2004 and 2006), a large population-based birth cohort in Rotterdam, the Netherlands. Questionnaires administered prenatally assessed demographic and lifestyle characteristics and maternal diet. Linear mixed models, with adjustment for relevant covariates, were used to estimate associations between the potential exposure determinants and DAP metabolite concentrations expressed as molar concentrations divided by creatinine levels.

Results: The median DAP metabolite concentration was 311 nmol/g creatinine for the first trimester, 317 nmol/g creatinine for the second trimester, and 310 nmol/g creatinine for the third trimester. Higher maternal age, married/living with a partner, underweight or normal weight (BMI of <18.5 and 18.5–<25), high education, high income, and non-smoking were associated with higher DAP metabolite concentrations, and DAP metabolite concentrations tended to be higher during the summer. Furthermore, fruit intake was associated with increased DAP metabolite concentrations. Each 100 g/d difference in fruit consumption was associated with a 7% higher total DAP metabolite concentration across pregnancy. Other food groups were not associated with higher DAP metabolite concentrations.

Conclusions: The DAP metabolite concentrations measured in urine of pregnant women in the Netherlands were higher than those in most other studies previously conducted. Fruit intake was the main dietary source of exposure to OP pesticides in young urban women in the Netherlands. The extent to which DAP metabolite concentrations reflect exposure to the active parent pesticide rather than to the less toxic metabolites remains unclear. Further research will be undertaken to investigate the possible effects of this relatively high-level OP pesticide exposure on offspring health.

Introduction

In the Netherlands more than 50% of the total surface area is used for agriculture purposes.[1] Organophosphate (OP) pesticides are a class of insecticides that are commonly used in agriculture. Between 1998 and 2008, approximately 35% of the insecticides used in the Netherlands were OP pesticides,[2] which may lead to high background exposure.

For non-occupationally exposed individuals, the exposure occurs most likely through the ingestion of food.[3] Further, residential exposure can occur through the use of insecticides in and around the house.[4-7] Exposures to high doses of OP pesticides are known to be neurotoxic in humans and animals.[8-10] Nevertheless, results obtained from both animal and human studies raise concerns about the potential health impact of low-level OP pesticide exposure in the general population.[11]

Animal studies have demonstrated that OP pesticide exposure levels even below the threshold for acetylcholinesterase inhibition can alter psychological disorder related gene expression,[12] induce changes in behavior and neurochemistry,[13] and result in cognitive impairments.[14,15] Moreover, low level OP pesticide exposure can change neuronal cell development,[16] induce oxidative stress,[17,18] and influence the thyroid hormone levels and the reproductive system.[19-21]

Fetuses and children are more susceptible to neurotoxic effects than adults as the human brain is particularly vulnerable during maturational and developmental processes.[22] Prenatal exposure to OP pesticides is potentially harmful because OP pesticides are able to cross the blood-brain barrier. Also, OP pesticides can cross the placental barrier, as they have been found in human amniotic fluid samples.[23] Further, epidemiological studies suggest that prenatal exposure to OP pesticides may be associated with adverse neurodevelopmental and birth outcomes,[24,25] although results are not conclusive.[26]

After absorption, most OP pesticides undergo bioactivation, during which the toxic oxon form is formed, followed by detoxification, which produces up to six dialkyl phosphate (DAP) metabolites.[27,28] These DAP metabolites have a short half-life and are mostly excreted in urine within 24 h.[29] As these DAP metabolites can stem from more than one OP pesticide, DAP metabolites are non-specific biomarkers of OP pesticides. Therefore, urinary DAP metabolite concentrations provide information about the total exposure to several parent OP pesticides.[30]

Several studies investigating prenatal OP pesticide exposure have observed that maternal characteristics, such as education, smoking, social economic status (SES), body mass index (BMI), and diet (especially the consumption of fruits and vegetables) are associated with

DAP metabolite concentrations in urine.[31-34] This was confirmed in two pilot studies in the Netherlands, both embedded in the Generation R Study. Moreover, the reported DAP metabolite concentrations were relatively high as compare to other birth cohort studies.[35,36]

Although several studies investigated the possible determinants of prenatal DAP metabolite concentrations in non-occupationally exposed individuals, several gaps remain. To the best of our knowledge only one study with a large sample size have jointly tested the different determinants of DAP metabolite concentrations to investigate what the main source of OP pesticide exposure in pregnant women is.[32] In contrast, most other studies relating dietary intake and other determinants to DAP metabolite concentrations used bivariate models wherein each possible predictor was tested separately.[31,33,34] Moreover, several studies, including our pilot study, investigated only broad food group categories[34,35] while few studies explored specific food items (e.g., apples).[32,33] The sample size of most studies limited the ability to test specific determinants of DAP metabolite concentrations.[31,34,35] It therefore remains unclear which determinants, food groups, and corresponding food items contribute most to the exposure. Large biomonitoring studies with detailed exposure history are needed to address this since such information is important for public health measures.

The Generation R cohort provides suitable data to determine the levels of prenatal DAP metabolite concentrations because of the large sample size, availability of three repeated urinary specimens across pregnancy, and the availability of detailed information of potential environmental determinants. Therefore, the objectives of the present study were to investigate the levels of DAP metabolites concentrations across pregnancy and to examine various determinants of DAP metabolite concentrations.

Methods

Study population and follow-up

The Generation R Study is a prospective population-based birth cohort designed to identify early environmental and genetic determinants of normal and abnormal development and health from fetal life onwards.[37] Mothers, who had a delivery date from April 2002 to January 2006 and lived in the study area in Rotterdam, the Netherlands, were qualified for inclusion and enrolled during pregnancy. The study protocol underwent human subjects review at Erasmus Medical Center, Rotterdam, the Netherlands and all participants provided written informed consent.

In total, 8879 mothers were enrolled during pregnancy. Of those, 4918 were enrolled during pregnancy from February 2004 to January 2006, when up to three spot urine

specimens were collected at the time of routine ultrasound examinations (<18, 18–25, >25 weeks of gestational age, respectively). A complete set of three urine specimens was available for 2083 pregnant women. We selected samples based on available follow-up data, which was obtained in 1449 children of these women. The availability of follow-up data was a priority for future studies on the possible associations between prenatal OP pesticide exposure and health related outcomes in children. In total, 800 women were randomly selected to determine the DAP metabolite concentrations in the maternal urine samples. Due to insufficient urine specimens, maternal DAP results were available for 778 complete urine sets and 6 incomplete urine sets (5 women with 2 samples and 1 woman with 1 sample).

Urine collection and analysis of DAP metabolites

Details of maternal urine specimen collection have been described elsewhere.[38] Briefly, all urine samples were collected between 8 am and 8 pm in 100 mL polypropylene urine collection containers that were kept for a maximum of 20 h in a cold room (4 °C) before being frozen at –20 °C in 20 mL portions in polypropylene vials. Measurements of six non-specific DAP metabolites of OP pesticides were conducted at Institut National de Santé Publique in Quebec (INSPQ), Canada, using gas chromatography coupled with tandem mass spectrometry (GC–MS/MS).[39]

Three dimethyl (DM) metabolites (dimethylphosphate (DMP), dimethylthiophosphate (DMTP), and dimethyldithiophosphate (DMDTP)) and three diethyl (DE) metabolites (diethylphosphate (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP)) were determined. DM metabolites are only generated by dimethyl OP pesticides, whereas DE metabolites are only generated by diethyl OP pesticides. The molar sum of DE and DM metabolite concentrations represents the total urinary DAP metabolite concentrations. Most OP pesticides degrade to form DAP metabolites. However, several OP pesticides do not degrade to form a DAP metabolite (e.g., Acephate). Therefore, the total DAP metabolite concentrations provide information about the total exposure to OP pesticides that generate DAP metabolites.[30]

The limits of quantification (LOQ) were 0.87 µg/l for DMP, 1.33 for DMTP, 0.30 for DMDTP, 1.67 for DEP, 0.40 for DETP, and 0.20 for DEDTP. The limit of detection (LOD) was 0.26 µg/l for DMP, 0.40 for DMTP, 0.09 for DMDTP, 0.50 for DEP, 0.12 for DETP, and 0.06 for DEDTP. The inter-day precision of the method during this project, expressed as the coefficient of variation (CV) and measured with the inclusion of the values <LOD, varied between 4.2–8.8% for DEDTP, 4.1–7.2% for DEP, 5.0–9.1% for DETP, 5.5–7.1% for DMDTP, 5.3–8.0% for DMP, and 5.5–7.7% for DMTP based on reference materials (clinical check-urine level II 637 E-495 and MRM E-459).

Molar concentrations were used to facilitate comparison of our results with those from other studies, based on the following molecular weights: DMP 126.0, DMTP 142.1, DMDTP 158.2, DEP 154.1, DETP 170.2, and DEDTP 186.2 g/mol. To account for urine dilution, the level of creatinine was determined in each sample based on the Jaffe reaction,[40] with a limit of detection of 0.28 mmol/l. The day-to-day precision for creatinine varied between 3.0 and 3.3 CV%.

To evaluate reliability of DAP metabolite measures, we made use of 45 participants included in the present study, which were also included in the pilot study,[35,36] resulting in two available DAP concentrations per sample. DAP metabolite concentrations in urine were, however, determined in two different laboratories, at the INSPQ in the present study and at the Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Germany in the pilot study. Intra class correlations (ICC) were calculated for the creatinine (g/l) and total DAP metabolite concentrations in nmol/L. The creatinine concentrations for the three trimesters had excellent ICC values (0.90–0.98) and the total DAP metabolite concentrations in nmol/L varied between good and excellent ICC values (0.81–0.95).[41] The median total DAP metabolite concentrations of the 45 overlapping participants from the current study tended to be slightly higher (median differences; <18 weeks = 65 nmol/L, 18–25 weeks = 50 nmol/L, and >25 weeks = 40 nmol/L).

Further, Pearson correlation coefficients were calculated to investigate whether the time elapsed between the date of sampling and the date of the analytical measurement had any influence on the DAP metabolite concentrations. The correlations were negligible and varied for the three measurements between –0.14 and 0.05.[42]

Determinants of OP pesticide exposure

Maternal demographic and lifestyle data were assessed by questionnaire or direct measurement during pregnancy. During early visits, data on maternal height and weight were measured and were used to calculate early BMI. Prenatal questionnaires were used to collect information about maternal age, parity, smoking (no smoking during pregnancy, smoked until pregnancy recognized, and continued smoking during pregnancy), alcohol intake during pregnancy (no alcohol consumption during pregnancy, alcohol consumption until pregnancy recognized, continued occasionally (<1 glass/week), and continued frequently (1+ glass/week)), marital status, highest completed education level (low: only lower vocational training, or <3 years at general secondary school; intermediate: 3+ years of secondary education, intermediate vocational training; high: university degree or higher vocational training), ethnicity (Dutch, other-western, and non-western), and household total net income (<1200 euro per month (i.e., below the Dutch social security level), 1200–2000 euro per month, and >2000 euro per month).

Data on potential occupational exposure to pesticides and pet ownership were also prenatally assessed by questionnaires. Pesticide exposure through pet ownership (dog, cat, or no pet) in the home might occur because flea treatments for cats and dogs (such as flea collars) may contain OP pesticides (e.g., Diazinon). Maternal occupational exposure to pesticides and partner's exposure to pesticides were prenatally determined by means of a questionnaire. To measure possible occupational exposure, the questions "do you work with pesticides?" and "does your partner work with pesticides?" were asked.

Maternal dietary intake in the first trimester was assessed using a modified version of a validated semi-quantitative food frequency questionnaire (FFQ).[43] The FFQ was administered at a median gestational age of 13.5 weeks (95% range 10.1–21.8 weeks) and covered the past three months. The FFQ includes questions on consumption frequency, portion sizes, and preparation methods of 293 food items and is structured according to meal patterns. The 293 food items were reduced to 24 predefined food groups (such as meat, grains, vegetables, fruits, etc.) according to the European Prospective Investigation into Cancer and Nutrition (EPIC)-soft classification, based on origin, culinary usage, and nutrient profiles.[44] Average daily energy intake was calculated using the Dutch food composition Table 2006. More details about the assessment of dietary intake are described elsewhere.[43] All food items were adjusted for energy intake (varying between 619 and 3452 kcal). Except for household income (13%), owning a dog (11%), owning a cat (11%), occupational exposure to pesticides (14%), partner's exposure to pesticides (31%), and maternal dietary determinants (22%), the percentage of missing values of these variables did not exceed 10%.

Statistical analysis

Urinary DAP concentrations were expressed on a volume (nmol/L) and creatinine basis (nmol/g creatinine). The three DM metabolites were summed as total DM and the three DE metabolites were summed as total DE. Total DAP concentrations were calculated by summing the six metabolites. Next, total DAP, DE, and DM metabolite concentrations were log10 transformed to achieve normal distributions.

Missing DAP metabolite (nmol/L) values at a specific time point were imputed 10 times with a multiple imputation method using other metabolite levels (nmol/L) from the same time point as predictors. Also, concentrations below the LOD were randomly assigned 10 imputed values below their LOD thresholds using a multiple imputation method.[45] Concentrations between LOQ and LOD were not imputed and kept for the analyses. To avoid loss of precision and power, missing values of potential confounding factors were also 10 times imputed with the use of a multiple imputation procedure.

We first provided descriptive statistics of the DAP metabolite concentrations in our study sample and compared those values with the values of several other studies that measured prenatal DAP metabolite concentrations. We then compared the median (P25, P75) maternal DAP metabolite concentrations by category of maternal characteristics and examined the association between these potential determinants and maternal urinary DAP metabolite concentrations with linear mixed model (LMM) analyses. LMM analyses allowed us to account for the repeated DAP metabolite concentrations within the same subject and to fit a correlation matrix on these repeated measurements. To explore the most important maternal characteristics of urinary DAP metabolite concentrations, we fitted a single LMM that included the maternal demographic and lifestyle determinants and season of urine collection as predictors and DAP metabolite concentrations across pregnancy as the outcome. We then used a stepwise variable selection procedure using the Akaike's information criterion (AIC) to identify the optimal model fit.

We also fitted a LMM to identify the most meaningful dietary intake predictors of maternal urinary DAP metabolite concentrations. We estimated the association between the various dietary intake categories and maternal urinary DAP concentrations across pregnancy for each food group separately. These associations were adjusted for the determinants identified by the AIC stepwise selection procedure. The food groups that had a statistically significant association ($P < 0.05$) with maternal urinary DAP concentrations across pregnancy were further examined by testing associations with specific food items from this group. Frequently consumed food items were expressed in 100 g/d. The food items that were not consumed by 20% of the participants were dichotomized (0 = no intake, 1 = intake).

Several sensitivity analyses were conducted. First, as the replacement of values below LOD with LOD/$\sqrt{2}$ is another common substitution method in environmental exposure studies,[46] we substituted values below LOD with LOD/$\sqrt{2}$ instead of using the MI method. Second, we reanalyzed the association between food group intake and DAP metabolite concentrations using only DAP concentrations from the <18 weeks of gestation period as the outcome because the FFQ was administered in the first trimester. Third, we reanalyzed the association between food groups and DAP metabolite concentrations including all food group variables in one model, thereby mutually adjusting the food groups for each other. Fourth, we modeled the most meaningful food intake predictors categorically (<50, 50–99, 100–149,150–199, and ≥200 g) instead of continuously to demonstrate the dose-response relationship. Fifth, we fitted models with metabolite concentrations expressed as nmol/L urine adjusted for creatinine concentration as a separate covariate.[47] Finally, we investigated whether the results were the same if missing confounder values were excluded rather than imputed. A p-value of <0.05 was defined as statistically significant. Statistical analyses were performed using SPSS (version 21) and R (version 3.2.3).[48]

Results

Sample characteristics

Most women were within the age category 30–<35 years (45.9%), had an early pregnancy BMI between 18.5 and <25 (65.9%), were nulliparous (62.3%), had a Dutch ethnic background (57.5%), and had a high educational background (54.9%) (Table 1). Moreover, most women were married or lived with a partner (89.7%), did not smoke during pregnancy (77.0%), and drank alcohol occasionally (less than 1 glass/week) during pregnancy (39.4%). Few women participating in this study worked with pesticides (0.6%) or had a partner that worked with pesticides (0.9%). A total of 7.4% of the women had a dog and 23.5% had a cat in their home. Selected participants in this study tended to be older, more frequently Dutch, more highly educated, from a household with higher income, and less likely to smoke during pregnancy than the overall cohort. The median DAP metabolite concentration in nmol/g creatinine across pregnancy was higher among those who were older, had a lower BMI, had a high income, higher education, did not smoke, and had partners (Table 1). Moreover, the median DAP metabolite concentrations in nmol/g creatinine across pregnancy was higher in the urine samples collected during the summer and among those who did not own a dog or a cat.

DAP metabolite levels in urine

Figure 1 presents descriptive statistics of the DAP metabolite concentrations in nmol/g creatinine by gestational period. Maternal urine specimens were collected on average (±SD) at 13.2 ± 1.8, 20.4 ± 0.9, and 30.4 ± 0.8 weeks of gestation. The median total DAP metabolite concentrations for <18, 18–25, and >25 weeks of gestation were 311, 317, and 310 nmol/g creatinine, respectively. The median DE metabolite concentrations measured at 18, 18–25, and >25 weeks of gestation (44, 43, and 42 nmol/g creatinine, respectively) were lower as compared to the median DM metabolite concentrations measured during the same gestational periods (245, 269, and 249 nmol/g creatinine, respectively). The DEDTP metabolite had a high percentage of values below the LOD in the three consecutive gestational periods (81%, 85%, and 85%, respectively). For the other five metabolites (DETP, DEP, DMDTP, DMP, and DETP) 80% or more of the concentrations were above the LOD.

The temporal variability of DAP concentrations in urine samples collected across pregnancy has been described in detail elsewhere.[35] Briefly, the total DAP metabolite concentrations across pregnancy showed weak to moderate correlations. The total DAP metabolites in nmol/L had an ICC of 0.43 (95%CI: 0.36–0.50) and the total DAP metabolites in nmol/g creatinine had an ICC of 0.51 (95%CI: 0.42–0.54).[41] Moreover, in accordance with the Pearson correlation coefficients, both the total DAP metabolite concentrations

Table 1. Demographic and lifestyle characteristics and residential and occupational exposure characteristics of 784 pregnant women from the Netherlands participating in the Generation R cohort and average DAP concentration in nmol/g creatinine by category of characteristics

Characteristic	Descriptive statistics		
	Generation R cohort (N=9778)	Included in the study (N=784)	DAP exposure [a] Median (P25, P75) (N=784)
Demographic and lifestyle characteristics at time of enrollment			
Age in years			
< 20	4.2 %	1.8%	292 (231, 382)
20-< 25	15.9 %	10.1%	329 (237, 453)
25-< 30	26.4 %	26.5%	323 (245, 481)
30-< 35	36.9 %	45.9%	381 (265, 517)
≥ 35	16.6 %	15.7%	382 (262, 484)
Missing, n	2	-	
BMI			
< 18.5	2.1 %	2.3%	371 (299, 561)
18.5-< 25	57.9 %	65.9%	375 (267, 507)
25-< 30	26.3 %	23.5%	342 (253, 449)
≥ 30	13.8 %	8.3%	263 (196, 432)
Missing, n	899	4	
Height in cm (quartiles)			
< 161	23.6 %	18.0%	341 (257, 499)
161 – < 168	27.4 %	35.8%	348 (246, 483)
168 – < 173	24.6 %	24.3%	366 (239, 492)
≥ 173	24.4 %	22.0%	365 (278, 503)
Missing, n	934	1	
Parity (Previous births)			
0	55.1 %	62.3%	362 (256, 502)
1	30.2 %	26.7%	376 (267, 502)
≥ 2	14.7 %	11.0%	280 (204, 426)
Missing, n	378	4	
Ethnicity			
Non-Western	38.4 %	29.8%	340 (243, 519)
Other Western	11.6 %	12.6%	334 (258, 484)
Dutch	50.0 %	57.5%	369 (256, 484)
Missing, n	694	-	
Education			
Low	26.5 %	14.9%	290 (199, 436)
Intermediate	30.7 %	30.2%	334 (242, 483)
High	42.8 %	54.9%	382 (279, 436)
Missing, n	1221	25	
Household income in euro's			
<1200 per month	20.7 %	12.6%	304 (219, 465)
1200–2000 per month	18.5 %	16.6%	319 (246, 465)
> 2000 per month	60.8 %	70.8%	379 (272, 497)
Missing, n	3066	102	

Continue

Continued

Characteristic	Descriptive statistics		
	Generation R cohort (N=9778)	Included in the study (N=784)	DAP exposure [a] Median (P25, P75) (N=784)
Marital status			
Married/ living with partner	85.5 %	89.7%	368 (266, 503)
No partner	14.5 %	10.3%	256 (187, 386)
Missing, n	1213	29	
Smoking			
No smoking during pregnancy	73.4 %	77.0%	372 (266, 506)
Until pregnancy recognized	8.6 %	8.9%	338 (258, 499)
Continued during pregnancy	18.0 %	14.1%	274 (181, 434)
Missing, n	1534	63	
Alcohol consumption			
No consumption during pregnancy	48.0 %	36.7%	328 (243, 484)
Until pregnancy recognized	13.2 %	17.5%	372 (266, 499)
Continued occasionally	31.6 %	39.4%	380 (255, 507)
Continued frequently	7.2 %	6.5%	346 (293, 435)
Missing, n	1870	40	
Season of urine collection			
Fall	-	22.1%	302 (186, 457)
Winter	-	21.2%	315 (198, 491)
Spring	-	29.0%	311 (199, 497)
Summer	-	27.8%	318 (205, 532)
Missing, n	-	7	
Work with pesticides			
Do not know	1.9%	2.1%	337 (242, 635)
No	97.4%	97.3%	363 (258, 495)
Yes	0.7%	0.6%	203 (167, 278)
Missing, n	3295	106	
Partner works with pesticides			
No	98.6%	99.1%	372 (263, 495)
Yes	1.4%	0.9%	243 (219, 596)
Missing, n	4952	243	
Owning a dog			
No, due to allergy	12.8%	11.9%	315 (249, 495)
No	77.9%	80.7%	375 (259, 503)
Yes	9.3%	7.4%	283 (191, 394)
Missing, n	2366	85	
Owning a cat			
No, due to allergy	14.0%	13.3%	342 (262, 483)
No	64.2%	63.2%	371 (260, 509)
Yes	21.8%	23.5%	329 (240, 478)
Missing, n	2404	83	

a. Median (P25, P75) DAP metabolite exposure concentrations are based on the averaged DAP metabolite concentrations across pregnancy (measured at three time points) in nmol/g creatinine for the study sample (n=784).

Metabolite	%<LOD	GM	min	P50	max
DEDTP					
< 18 weeks	81%	0.15	0.05	0.37	130.61
18-25 weeks	85%	0.05	0.06	0.37	113.24
>25 weeks	85%	0.02	0.04	0.34	102.69
DETP					
< 18 weeks	12%	6.16	0.25	7.58	613.43
18-25 weeks	12%	6.08	0.17	7.13	409.68
>25 weeks	12%	5.65	0.24	7.32	461.09
DEP					
< 18 weeks	3%	31.69	2.95	32.29	2987.04
18-25 weeks	5%	30.81	2.43	31.11	467.16
>25 weeks	4%	30.22	1.82	31.08	485.68
Total DE [a]					
< 18 weeks		43.58	3.45	43.89	3030.49
18-25 weeks		41.48	3.35	42.63	660.48
>25 weeks		40.93	2.85	42.07	745.13
DMDTP					
< 18 weeks	20%	2.31	0.10	3.12	450.78
18-25 weeks	18%	2.89	0.16	3.37	165.08
>25 weeks	18%	2.47	0.16	2.98	270.65
DMTP					
< 18 weeks	3%	85.02	1.01	102.47	4237.91
18-25 weeks	4%	96.16	2.63	110.36	1717.22
>25 weeks	2%	92.73	1.99	102.93	1889.13
DMP					
< 18 weeks	-	129.78	3.83	129.77	1547.11
18-25 weeks	-	136.05	12.31	136.54	1528.82
>25 weeks	-	125.69	7.12	128.73	1656.09
Total DM [b]					
< 18 weeks		249.46	6.64	244.77	6106.54
18-25 weeks		263.69	24.81	268.94	2468.64
>25 weeks		247.34	12.30	249.00	2908.10
Total DAP [c]					
< 18 weeks		311.24	15.43	310.91	6445.29
18-25 weeks		319.74	41.04	316.77	3069.60
>25 weeks		302.48	21.08	309.85	3013.83

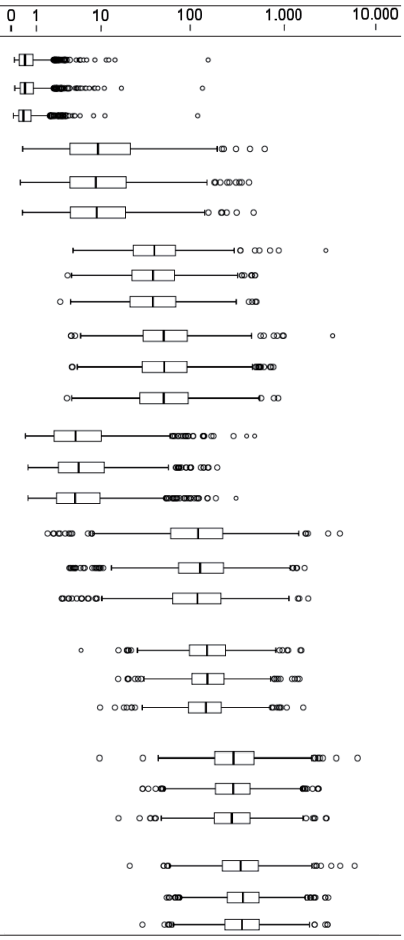

Figure 1. Descriptive statistics of DAP metabolite concentrations in nmol/g creatinine from 784 pregnant women from the Netherlands participating in the Generation R cohort.
Note. N=784. Concentrations below the limit of detection (LOD) were randomly assigned imputed values below their LOD thresholds using a multiplicative lognormal imputation method (Palarea-Albaladejo & Martin-Fernandez, 2015).
a. Diethyl alkyl phosphates is the sum of DEDTP, DETP and DEP.
b. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP and DMP.
c. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.

in nmol/L (r = 0.14–0.24) and in nmol/g creatinine (r = 0.17–0.34) across pregnancy, showed weak correlations.[42]

Predictors of urinary OP pesticide metabolite levels

Maternal demographic and lifestyle characteristics

Table 3 presents the maternal demographic and lifestyle determinants of total DAP, DM, and DE metabolite concentrations. Maternal age was positively associated with total DAP and DE urinary metabolite concentrations. A one year higher maternal age was associated with a 1% (95%CI: 0–2%) increase in total DAP and a 1% (95%CI: 0–2%) increase in DE urinary metabolite concentrations. Women with a BMI of 25–< 30 had 10% (95%CI: 1–20%) lower total DAP, 9% (95%CI: 1–19%) lower DM, and 14% (95%CI: 3–26%) lower DE metabolite concentrations as compared to women with a BMI 18.5–< 25. Also, women with a BMI of ≥30 had 24% (95%CI: 9–41%) lower total DAP, 23% (95%CI: 7–40%) lower DM, and 45% (95%CI: 24–70%) lower DE metabolite concentrations as compared to women with a BMI 18.5–< 25.

Further, women with a high maternal educational attainment had 15% (95%CI: 2–30%) higher total DAP and 17% (95%CI: 4–33%) higher DM metabolite concentrations than women with a low educational attainment. Compared to women with a low household income, women with a high household income had 29% (95%CI: 9–52%) higher DE metabolite concentrations. Next, women with a non-western ethnicity had 10% (95%CI: 1–21%) higher total DAP and 15% (95%CI: 5–26%) higher DM metabolite concentrations compared to Dutch women.

Moreover, women who did not smoke during pregnancy had 23% (95%CI: 10–38%) higher total DAP, 21% (95%CI: 8–36%) higher DM, and 37% (95%CI: 20–57%) higher DE metabolite concentrations than women who continued smoking during their pregnancy. Similarly, women who smoked only until the pregnancy was recognized had 26% (95%CI: 9–47%) higher total DAP, 24% (95%CI: 7–45%) higher DM, and 38% (95%CI: 16–65%) higher DE metabolite concentrations than women who continued smoking during their pregnancy. Differences in total DAP, DM, and DE metabolite concentrations were observed between the seasons of urine collection. The urine samples collected during the summer contained 11% (95%CI: 3–20%) more DAP and 16% (95%CI: 7–26%) more DM metabolite concentrations than the urine samples collected during the fall. Urine samples collected during the winter had 11% (95%CI: 2–21%) lower DM metabolite concentrations than the concentrations collected during the summer, but 14% (95%CI: 3–25%) higher DE metabolite concentrations than the urine samples collected during the spring.

Table 3. Multivariable determinants of dialkyl phosphates metabolite concentrations on a creatinine basis (nmol/g creatinine) across pregnancy among 784 women participating in the Generation R cohort

	Total dialkyl phosphates [a]		Dimethyl alkyl phosphates [b]		Diethyl alkyl phosphates [c]	
Determinants	B (95%CI)	P	B (95%CI)	P	B (95%CI)	P
Age in years	0.004 (0.001 to 0.008)	0.048*	0.003 (-0.001 to 0.007)	0.099	0.006 (0.001 to 0.010)	0.020*
Marital status						
Married/ partner	0.098 (0.045 to 0.151)	<0.001*	0.107 (0.051 to 0.162)	<0.001*	-	
No partner	ref		ref		-	
BMI						
<18.5	0.036 (-0.063 to 0.135)	0.481	0.035 (-0.069 to 0.139)	0.509	0.015 (-0.108 to 0.139)	0.808
18.5-<25	ref		ref		ref	
25-<30	-0.042 (-0.078 to -0.006)	0.023*	-0.039 (-0.077 to -0.002)	0.040*	-0.055 (-0.100 to -0.011)	0.014*
≥30	-0.093 (-0.149 to -0.036)	0.001*	-0.088 (-0.146 to -0.029)	0.003*	-0.162 (-0.230 to -0.094)	<0.001*
Parity						
0	0.085 (0.033 to 0.137)	0.001*	0.073 (0.019 to 0.127)	0.008*	0.119 (0.056 to 0.182)	<0.001*
1	0.059 (0.006 to 0.113)	0.031*	0.054 (-0.002 to 0.110)	0.059	0.077 (0.011 to 0.143)	0.022*
≥2	ref		ref		ref	
Education						
high	0.061 (0.007 to 0.115)	0.027*	0.070 (0.015 to 0.125)	0.013*	-	
medium	0.033 (-0.016 to 0.082)	0.190	0.041 (-0.009 to 0.092)	0.110	-	
low	ref		ref		-	
Income						
high	-		-		0.111 (0.039 to 0.183)	0.003*
medium	-		-		0.072 (-0.003 to 0.146)	0.059
low	-		-		ref	
Ethnicity						
Non-Western	0.043 (0.005 to 0.082)	0.028*	0.060 (0.021 to 0.100)	0.003*	-	
Other Western	-0.021 (-0.068 to 0.025)	0.375	-0.017 (-0.066 to 0.031)	0.486	-	
Dutch	ref		ref		ref	

Continue

Continued

	Total dialkyl phosphates [a]		Dimethyl alkyl phosphates [b]		Diethyl alkyl phosphates [c]	
Determinants	B (95%CI)	P	B (95%CI)	P	B (95%CI)	P
Smoking						
no smoking during pregnancy	0.091 (0.043 to 0.140)	<0.001*	0.083 (0.033 to 0.133)	0.001*	0.138 (0.079 to 0.197)	<0.001*
Until pregnancy recognized	0.102 (0.038 to 0.167)	0.002*	0.094 (0.028 to 0.161)	0.006*	0.141 (0.064 to 0.218)	<0.001*
continued during pregnancy	ref		ref		ref	
Work with pesticides						
Do not know	0.251 (0.015 to 0.487)	0.037*	-		0.395 (0.104 to 0.685)	0.008*
No	0.180 (-0.027 to 0.389)	0.089	-		0.279 (0.022 to 0.536)	0.033*
Yes	ref		-		ref	
Season						
Autumn	-0.046 (-0.080 to -0.011)	0.009*	-0.064 (-0.100 to -0.028)	0.001*	0.043 (-0.001 to 0.087)	0.052
Winter	-0.033 (-0.069 to 0.003)	0.070	-0.047 (-0.084 to -0.009)	0.017*	0.055 (0.013 to 0.097)	0.011*
Spring	-0.020 (-0.051 to 0.011)	0.210	-0.022 (-0.055 to 0.011)	0.187	ref	
Summer	ref		ref		0.018 (-0.021 to 0.057)	0.368
Dog ownership						
No due to allergy	0.063 (-0.009 to 0.135)	0.089	-		0.059 (-0.030 to 0.148)	0.191
No	0.063 (0.001 to 0.125)	0.047*	-		0.078 (0.003 to 0.153)	0.041*
Yes	ref		-		ref	

a. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.
b. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP and DMP.
c. Diethyl alkyl phosphates is the sum of DEDTP, DETP and DEP.
* $p<0.05$.

Table 4. Associations [a] between food groups per 100g/d and dialkyl phosphates metabolite concentrations on a creatinine basis (nmol/g creatinine) across pregnancy among 610 pregnant women participating in the Generation R cohort

	Total dialkyl phosphates [b]		Dimethyl alkyl phosphates [c]		Diethyl alkyl phosphates [d]	
Food intake	B (95%CI)	P	B (95%CI)	P	B (95%CI)	P
Per 100g/d						
Vegetables	0.001 (-0.028 to 0.030)	0.943	-0.007 (-0.037 to 0.022)	0.629	0.026 (-0.010 to 0.062)	0.154
Fruits	0.030 (0.016 to 0.045)	<0.001*	0.030 (0.015 to 0.046)	<0.001*	0.031 (0.013 to 0.049)	0.001*
Nuts	0.078 (-0.114 to 0.270)	0.462	0.091 (-0.109 to 0.291)	0.374	0.085 (-0.154 to 0.323)	0.487
Dairy	-0.003 (-0.011 to 0.005)	0.428	-0.004 (-0.012 to 0.004)	0.389	-0.002 (-0.012 to 0.007)	0.657
Fish	0.120 (-0.003 to 0.244)	0.056	0.113 (-0.016 to 0.242)	0.085	0.064 (-0.089 to 0.217)	0.415
Grain	-0.006 (-0.037 to 0.024)	0.692	-0.016 (-0.047 to 0.016)	0.330	0.018 (-0.019 to 0.056)	0.341
Meat	-0.031 (-0.079 to 0.017)	0.205	-0.029 (-0.079 to 0.021)	0.252	-0.035 (-0.094 to 0.025)	0.252

a. Adjusted for energy intake, maternal age, BMI categories (>18,5, 18,5-25, 25-30, 30+), parity categories (0,1,2+), ethnicity categories (Dutch, other-western, non-western), education categories (low, intermediate, high), household income categories(<1200 per month, 1200–2000 per month > 2000 per month), marital status, smoking categories (no smoking during pregnancy, smoked until pregnancy was known, smoked during pregnancy), and season of urine collection (fall, winter, spring, summer).

b. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.

c. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP and DMP.

d. Diethyl alkyl phosphates is the sum of DEDTP, DETP and DEP.

* $p<0.05$.

Residential determinants

No consistent associations between pet ownership (cat and dog) and DAP metabolite concentrations were observed. For example, we observed that participants who did not own a dog had 16% (95%CI: 0 to 33%) higher DAP and 20% (95%CI:1–42%) higher DE metabolite concentrations.

Maternal dietary determinants

Table 4 presents the adjusted associations between consumption of food groups and total DAP, DM, and DE metabolite concentrations. The consumption of fruit was associated with total DAP metabolite concentrations, DM metabolite concentrations, and DE metabolite concentrations. A 100 g/d increase in consumption of fruits was associated with a 7% (95%CI: 4–11%) increase in DAP metabolite concentrations, a 7% (95%CI: 4–11%) increase in DM metabolite concentrations, and a 7% (95%CI: 3–12%) increase in DE metabolite concentrations. There were no statistically significant associations between the consumption of vegetables, nuts, dairy, fish, grain, and meat with total DAP, DM, and DE metabolite concentrations ($P > 0.05$).

Table 5 presents the adjusted associations between the consumption of different fruit types and total DAP, DM, and DE metabolite concentrations. The consumption of oranges/grapefruits and apples were associated with total DAP metabolite concentrations and DM metabolite concentrations. A 100 g/d higher consumption of oranges/grapefruits was related to a 13% (95%CI: 3–24%) higher total DAP metabolite concentration and a 14% higher DM metabolite concentration (95%CI: 3–26%). A 100 g/d higher apple consumption was associated with a 14% (95%CI: 4–26%) higher total DAP metabolite concentration and a 16% (95%CI: 5–29%) higher DM metabolite concentration. Further, women who consumed apricots and grapes/cherries also had significantly higher DAP metabolite and DM metabolite concentrations compared to women who did not consume these fruits. Consumers of lemons/limes, apricots, kiwis, strawberries/raspberries, mangos and pineapples/melons had higher DE metabolite concentrations than women who did not consume these fruits.

Sensitivity analysis

The results were consistent when the LOD/$\sqrt{2}$ substitution method was used (see Tables S1, S2, and S3). As part of the sensitivity analysis, we tested the association between food groups (and fruit types) and DAP metabolite concentrations, only using the measurement from the <18 weeks of gestation period as an outcome (see Tables S4 and S5). The results were similar to the results presented earlier (see Table 4, Table 5), the consumption of fruits was significantly associated with total DAP, DM, and DE metabolite concentrations. Within fruit types, again apples, oranges/grapefruits, and apricots were significantly associated with DAP metabolite concentrations. Similar results were found when the

Table 5. Associations [a] between fruit groups per 100g/d and dialkyl phosphates metabolite concentrations on a creatinine basis (nmol/g creatinine) across pregnancy among 610 pregnant women participating in the Generation R cohort

	Total dialkyl phosphates [b]		Dimethyl alkyl phosphates [c]		Diethyl alkyl phosphates [d]	
Fruit intake	B (95%CI)	P	B (95%CI)	P	B (95%CI)	P
Mandarin	0.040 (-0.045 to 0.124)	0.359	0.045 (-0.044 to 0.133)	0.321	-0.018 (-0.123 to 0.087)	0.732
Orange/grapefruit	0.053 (0.012 to 0.094)	0.011*	0.057 (0.014 to 0.099)	0.009*	0.040 (-0.011 to 0.091)	0.120
Lemon/lime [e]	0.031 (-0.004 to 0.065)	0.080	0.028 (-0.008 to 0.064)	0.128	0.044 (0.001 to 0.087)	0.043*
Banana	0.011 (-0.052 to 0.074)	0.728	0.013 (-0.053 to 0.078)	0.707	0.021 (-0.056 to 0.099)	0.588
Kiwi [e]	0.021 (-0.018 to 0.059)	0.295	0.004 (-0.036 to 0.044)	0.846	0.079 (0.031 to 0.127)	0.001*
Apple	0.057 (0.015 to 0.099)	0.008*	0.065 (0.021 to 0.109)	0.004*	0.031 (-0.022 to 0.084)	0.247
Pear [e]	0.016 (-0.020 to 0.052)	0.395	0.011 (-0.027 to 0.049)	0.566	0.025 (-0.020 to 0.070)	0.272
Mango [e]	0.019 (-0.017 to 0.055)	0.303	0.014 (-0.024 to 0.051)	0.475	0.044 (-0.001 to 0.089)	0.056
Avocado [e]	-0.001 (-0.038 to 0.036)	0.950	-0.004 (-0.043 to 0.035)	0.837	-0.003 (-0.049 to 0.043)	0.901
Peach/nectarine [e]	0.017 (-0.018 to 0.052)	0.336	0.018 (-0.018 to 0.055)	0.330	0.026 (-0.018 to 0.069)	0.245
Apricot [e]	0.063 (0.018 to 0.108)	0.006*	0.064 (0.017 to 0.111)	0.007*	0.068 (0.012 to 0.124)	0.017*
Plum [e]	0.027 (-0.010 to 0.063)	0.153	0.024 (-0.014 to 0.062)	0.207	0.037 (-0.008 to 0.083)	0.107
Strawberry/raspberry [e]	0.008 (-0.029 to 0.045)	0.664	-0.002 (-0.041 to 0.036)	0.904	0.050 (0.004 to 0.096)	0.033*
Grape/cherry [e]	0.052 (0.015 to 0.088)	0.005*	0.054 (0.016 to 0.092)	0.006*	0.042 (-0.004 to 0.087)	0.072
Pineapple/melon [e]	0.015 (-0.021 to 0.051)	0.415	0.007 (-0.031 to 0.044)	0.727	0.053 (0.009 to 0.097)	0.019*
Canned fruit [e]	0.005 (-0.036 to 0.046)	0.811	0.003 (-0.040 to 0.046)	0.882	0.028 (-0.023 to 0.079)	0.289

a. Adjusted for energy intake, maternal age, BMI categories (>18,5, 18,5-25, 25-30, 30+), parity categories (0,1,2+), ethnicity categories (Dutch, other-western, non-western), education categories (low, intermediate, high), household income categories(<1200 per month, 1200–2000 per month > 2000 per month), marital status, smoking categories (no smoking during pregnancy, smoked until pregnancy was known, smoked during pregnancy), and season of urine collection (fall, winter, spring, summer).

b. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.

c. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP and DMP.

d. Diethyl alkyl phosphates is the sum of DEDTP, DETP and DEP.

e. intake versus no intake.

associations of food groups with DAP metabolite concentrations were mutually adjusted (see Table S6). Other sensitivity analyses also supported the consistency of the results. when modeled categorically, higher intake of fruit was associated with increased DAP metabolite concentrations (see Table S7). When the models were fitted with metabolite concentrations expressed as nmol/L and adjusted for creatinine by including creatinine as a covariate, results compared to Table 3 were mostly similar but slightly weaker. Moreover, BMI, maternal age, parity, and dog ownership no longer predicted total DAP metabolite concentrations (see Table S8). The results of this sensitivity analyses were similar with the primary analyses for the food groups, but slightly different for fruit types (see Table S9 and S10). When we fitted the models with metabolite concentrations expressed as nmol/L and adjusted for creatinine with the total DAP metabolite measurement from <18 weeks of gestation period as the outcome (see Tables S11 and S12), both food group and fruit type results with creatinine adjustment were similar to the results presented in Table 4, Table 5. But, the associations between fruit types and DE metabolite concentrations were weaker. Finally, the results were similar when we examined the association between possible determinants and DAP metabolite concentrations without participants with imputed covariate data (see Tables S13, S14, and S15).

Discussion

In this study we reported prenatal levels of DAP metabolite concentrations across pregnancy and identified determinants of prenatal exposure to OP pesticides (or their degradation products) in an urban population of Dutch pregnant women. Our results suggest that fruit intake was the main source of exposure. Furthermore, we observed seasonal variation in total DAP metabolite concentrations with the highest concentrations during the summer. Higher maternal age, married/living with a partner, underweight or normal weight (BMI of <18.5 and 18.5–<25), high education, high income, and non-smoking were associated with higher DAP metabolite concentrations. Pet ownership did not contribute to increased DAP metabolite concentrations.

These results extend those of Spaan et al. (2015) and Ye et al. (2008), who also observed relatively high levels of DAP metabolite concentrations among a subset of the Generation R Study population as compared to other American and European studies (Fig. 2). The median total DAP metabolite concentrations in this study (311 nmol/g creatinine, 224 nmol/L) was slightly higher than in two previous pilot studies of the Generation R cohort (215 nmol/g creatinine, 129 nmol/L). The median DAP metabolite concentrations in this study were approximately 3 times higher compared to the urinary DAP metabolite concentrations in pregnant women from the Canadian MIREC cohort (Median = 78 nmol/L), which used the same analytical lab (INSPQ,

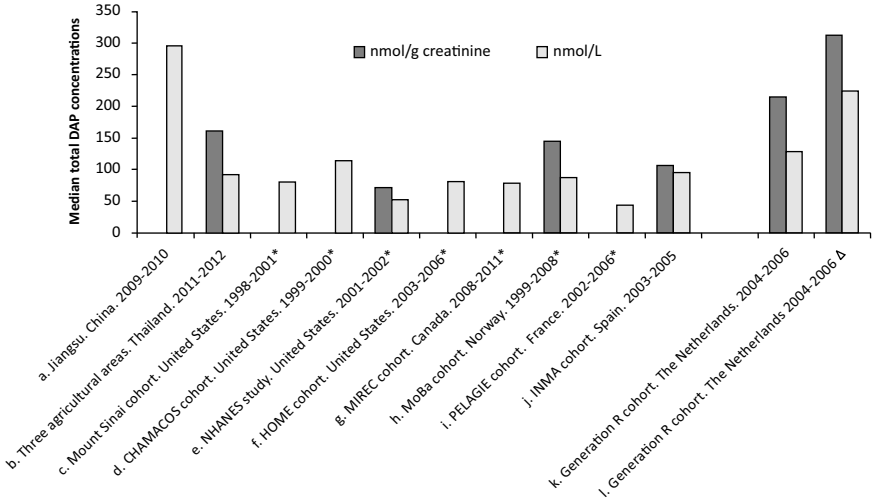

Figure 2. Comparison of total dialkyl phosphates (DAP) metabolite concentrations on a creatinine-basis (nmol/g creatinine) and a wet-weight metabolite basis (nmol/L) in maternal urine of various birth cohorts. *Geometric mean instead of the median DAP concentrations is presented.

a. Mother-infant pair cohort study from the Sheyang County, China. One spot urine sample collected prior to delivery from 310 mothers.[55]

b. Pregnant women sampled in hospitals from the Amnatchareon Province, Nakhonsawan Province and the Kanchanaburi Province of Thailand. One spot urine sample collected at 28 weeks of gestation from 86 women.[54]

c. Prospective cohort study in Mount Sinai Children's Environmental health center, New York City. Spot urine collected at mean gestational age 31.2 weeks from 285 to 297 women.[51]

d. Prospective cohort study in Salinas Valley, California. Two spot urine samples collected at baseline and 26 weeks of gestation. The geometric mean value represents the average of the two DAP metabolite concentrations.[49]

e. National Health and Nutrition Examination Survey study. A study representing the US population of all ages. One spot urine sample collected of 126 women during pregnancy.[50]

f. Prospective birth cohort in Cincinnati metropolitan area. Two spot urine samples collected at 16 and 26 of gestation from 344 women. The geometric mean value represents the average of the two DAP metabolite concentrations.[52]

g. Maternal-Infant Research on Environmental Chemicals study. One spot urine sample collected during the first trimester from 1884 women.[33]

h. A pregnancy cohort in Norway. Ten pools of one 1-ml urine samples from 11 women at 17 weeks of gestation.[50]

i. Mother child cohort in Brittany, France. One spot urine sample collected at <19 weeks of gestation from 231 women.[53]

j. INfancia y Medio Ambiente project (Environment and Childhood), to investigate the effects of environmental exposure, diet and genetics on fetal and child development. One spot urine sample collected at the third trimester from 573 women (mean = 32.2 weeks of gestagion).[32]

k. A prospective population-based birth cohort in Rotterdam, the Netherlands. Designed to identify the early environmental and genetic determinants of normal and abnormal development and health from fetal life onwards. Data combined from two previous small pilot studies. One to three spot urine samples collected at <18 weeks, 18–25 weeks and >25 weeks of gestation from 168 women. The values represent the average of the three median DAP metabolite concentrations.[35,36]

l. Δ Current study. Three spot urine samples collected at <18 weeks, 18–25 weeks, and >25 weeks of gestation from 784 women. The values represent the average of the three median DAP metabolite concentrations.

Quebec) as this study.[33] Moreover, the DAP metabolite concentrations were higher than the urinary DAP metabolite concentrations from pregnant women of several American studies (CHAMACOS cohort: median = 115 nmol/L, NHANES study: median = 72 nmol/g creatinine, 52 nmol/L, Mount Sinai cohort: 82 nmol/L, and HOME cohort: median = 81 nmol/L),[49-52] European studies (France PELAGIE cohort: median = 44 nmol/L, Norwegian MoBa cohort: GM = 145 nmol/g creatinine, 87 nmol/L, and Spanish INMA cohort: GM = 107 nmol/g creatinine, 96 nmol/L),[32,50,53] and compared to a study from Thailand (median = 161 nmol/g creatinine, 90 nmol/L.[54] In contrast, the DAP metabolite concentrations from our study were considerably lower than those observed in China (median = 296 nmol/L).[55]

The results of our study suggest that the relatively high-level exposure to OP pesticides or their degradation products among this general population cohort in the Netherlands may be related to the high consumption of fruits. Although results must be compared carefully since different methods were used to measure diet, the fruit and vegetable intake of our study sample in the Dutch population (median of 295 g/day) was higher compared to the fruit and vegetable intake of NHANES subjects (median of 167 g/day), who were women of reproductive age.[56,57]

Another reason that might explain the differences in DAP metabolite concentrations between the various studies of pregnant women are differences in population characteristics. Our study population consisted mainly of well-educated women with a relatively high family income. Compared to our study, in both the CHAMACOS and the Mount Sinai Hospital birth cohorts lower levels of DAP metabolite concentrations were measured among their populations, which both include mainly participants of ethnic minorities and low SES. SES is known to be positively related with the consumption fruit and vegetables,[58] an important source of OP pesticide exposure.[3] This could also explain why SES-related population characteristics in our study, such as BMI, parity, marital status, and smoking status, were associated with DAP metabolite concentrations. However, when we controlled for fruit intake, the associations remained essentially unchanged. Married women in general make healthier food choices compared to non-married women[59,60] and dietary patterns are strongly related to SES, ethnic differences, and BMI.[58,61-63] However, compared to the Generation R cohort, both the MIREC and the PELAGIE cohort also comprised populations with high SES, yet considerably lower DAP metabolite concentrations were measured. Most likely the differences in DAP metabolite concentrations between cohorts cannot fully be explained by differences in SES.

In addition to the consumption of fruits, the dose of OP pesticides present in or on the fruits also determines the exposure levels. Possibly, the higher DAP metabolite levels in pregnant women are also due to the farming practices in the Netherlands. The Netherlands

uses more pesticides and fertilizers per square km of farmland than most other Organization for Economic Co-operation and Development (OECD) countries, such as the United States and Canada.[64] Whether these intense farming activities increase the level of OP exposure through consumption of domestic fruits is unclear. However, between 1998 and 2008 approximately 1/3 of all insecticides used in the Netherlands were OP pesticides, with DM metabolite generating OP pesticides being the most frequently used. For example, in 2004 of all insecticides used in the Netherlands 32% were OP pesticides that generate DM metabolites (Dimethoate = 30%, Malathion = 0.9%, Parathion-methyl = 1.2% and pirimiphos-methyl = 0.4%) and only 0.075% were OP pesticides that generate DE metabolites (chlorpyrifos = 0.25% chlorfenvinphos = 0.5%).[2] This may also explain why in our study sample the DM metabolite concentrations were much more present in urine than the DE metabolite concentrations. Similarly, many previous studies also reported higher DM metabolite concentrations than DE metabolite concentrations.[32]

The DAP metabolite concentrations measured in our study however, were considerably lower compared to the levels observed in China and Taiwan.[55,65,66] This may be explained by China's heavy use of OP pesticides in agricultural activities.[67] The pesticide residues, often from OP pesticides, on agricultural products in Chinese markets are easily detected, with some of these products showing high levels of residues exceeding the safe standard.[68]

Our results are in agreement with findings obtained by Bradman et al. (2003), Llop et al. (2017), Lu et al. (2008), Sokoloff et al. (2016), and Yolton et al. (2013) who concluded that DAP metabolite levels in urine vary between seasons, and that diet, especially fruit, was associated with OP pesticide exposure. More specifically, Sokoloff et al. (2016) found citrus fruits and apple juice intake to be related to higher DAP metabolite concentrations and Llop et al. (2017) found that apples/pears and stone fruits intake were associated with increased DAP metabolite concentrations. Our result in which the total DAP and DM metabolite concentrations were higher in summer than in other seasons might be explained by the increased fruit consumption during the summer. Although not statistically significant, in our study women consumed more fruit during the summer than during other seasons.

The observation that dietary intake of fruits was the main source of exposure is in line with the observation that a large fraction of fruits have detectable OP pesticide residue levels.[69,70] Specific information on the presence of OP pesticide residues on fruit can be retrieved from the Quality Programme for Agricultural Products (KAP) database in the Netherlands.[69] Detectable residues from 18 different OP pesticides were found on fruit samples tested between 2004 and 2006 for pesticides. For example, 45 (35%) out of the 130 apples tested positive for azinphos-methyl residues. Whether OP pesticides that generate DM metabolites are more commonly applied to certain fruits and OP pesticides

that generate DE metabolites to other fruits is unclear. However, out of all the samples that tested positive for OP pesticide residues in the KAP database, the OP pesticides that generate DM metabolites (e.g., Dimethoate, Azinphos-methyl, and Malathion) were more frequently detected on apples (68%), oranges/grapefruits (64%), and grapes/cherries (61%) than OP pesticides that generate DE metabolites (e.g., Chlorpyrifos, Diazinon, and Ethion).[69] This is in line with our observation that the intake of these fruits were positively associated with DM metabolite concentrations and not with DE metabolite concentrations. Caution is needed here since the intake of lemons/limes was positively associated with DE metabolite concentration, but residues of DM metabolite generating OP pesticides were more frequently detected on lemons/limes (55%). Moreover, only few residues of both DM and DE metabolite generating OP pesticides were detected on kiwis, strawberries, and pineapples/melons.[69]

Although we did not measure the residential use of pest control items, it has been suggested that pet ownership might result in increased DAP metabolite concentrations because flea control items may contain OP pesticides.[5] For example, in the Netherlands flea collars for both cats and dogs from the brand Beaphar contain the OP pesticide Diazinon. However, no increased DAP metabolite concentrations were observed in our study in participants with cats or dogs.

Our finding of a lower DAP metabolite concentration in smokers could be explained by reduced fruit intake. Women who smoked during pregnancy had significantly lower fruit intake than those who did not smoke during pregnancy. However, It has also been suggested that nicotine intake may influence the metabolism and toxicity of OP pesticides.[71] An animal study showed that nicotine exposure could alter OP metabolism and that the extent of brain acetylcholinesterase (AChE) inhibition was reduced due to the co-exposure of OP pesticides and nicotine.[71] The observed differences in DAP metabolite concentrations across BMI categories could also be explained by diet and SES although we adjusted for these variables. Yet, it may also be that women with higher BMI will excrete more creatinine,[72] and thus their DAP concentrations on a nmol/g creatinine basis will appear lower. Some support for this explanation comes from the sensitivity analyses. When we fitted the models with metabolite concentrations expressed as nmol/L and adjusted for creatinine, the effect of BMI on total DAP and DM metabolites concentrations were attenuated.

Our study has a few limitations that need to be considered. DAP metabolites can also be found in food products and the environment due to environmental degradation.[73,74] The extent to which DAP metabolite concentrations reflect exposure to the active parent pesticide rather than to less toxic metabolites remains therefore unclear.[75] Nevertheless, the

measurement of DAP concentrations is scientifically accepted as a useful tool to identify and compare degrees of OP pesticide exposure in diverse populations.[76]

Further, our results suggest that the main route of exposure was through the ingestion of fruits. Rather than only rely on DAP metabolites, which reflect the total exposure to most OP pesticides[30] through all exposure routes (dermal, inhalation, ingestion) in combination with questionnaire information, it would also have been interesting to verify exposure by taking environmental and dietary samples such as fruit.

Additionally, we did not have information whether our participants consumed organic food products. Several studies have shown that individuals following an organic diet have significantly lower DAP metabolite concentrations in their urine than individuals with a non-organic diet.[77-79] It would have been informative to have data on the type of diet (organic or non-organic) to test whether organic food consumption could act as an effect modifier of the relation between fruit intake and DAP metabolite concentrations.

Moreover, we were not able to assess residential exposure to OP pesticides in detail. Besides the question about pet ownership, we did not have any information about possible other residential pesticide use in and around the home by the participant, another household member, or a professional exterminator. It would have been informative to investigate whether participants used residential products, which may contain OP pesticides such as insecticides for the lawn and garden (e.g., emulsifiable concentrate), insecticides for house plants, and residential pest products (e.g., fly control insecticides and moth killer cassettes). Also, it would have been informative to ask participants who owned a cat or a dog, whether they treated their pet with flea products.

Next, since dietary intake was not examined over time, we were not able to capture possible changes in diet as the pregnancy progressed. Therefore, the assumption was made that dietary intake during the first trimester of pregnancy reflects the dietary intake over the whole pregnancy period. Nevertheless, the associations between dietary intake and metabolite levels that were collected during the first trimester were similar to the associations between dietary intake and metabolite levels across pregnancy, which suggests that the observed associations are stable across weeks.

Another limitation of this study is the absence of information about the exact time of spot urine sampling. Because the urine spot samples were collected between 8am and 8pm, there may have been a combination of first morning and random spot samples. Concentrations of chemicals, urine volume and the rate of excretion vary with fluid intake, time of day, and other factors.[80-82] Although time of sample collection is unlikely to confound the association between possible determinants of OP pesticide exposure

and DAP metabolite concentrations, the difference in DAP metabolite concentrations between morning and random spot urine samples could have been tested as a predictor of DAP metabolite concentrations.

Moreover, this study was limited by the absence of information about the urine samples storage time in the 4 °C cold room as it might be plausible that OP pesticides degrade into DAP metabolites during such storage. It would have been informative to analyze whether storage time was associated with DAP metabolite concentrations to exclude the possibility that the samples had degraded during this period.

Furthermore, DAP metabolites are known to have a short half-life and are mostly excreted in urine within 24 h, which can result in day-to-day variability in exposure within subjects.[83] Ideally, many urine specimens need to be sampled during pregnancy. Our study includes three measures of DAP metabolite concentrations across pregnancy among a large sample which is more frequent than most other previous studies of prenatal OP exposure[24,32,33] and a key strength of this study.

Another strength of the study was the availability of urinary creatinine levels, which allowed us to adjust for urinary dilution. During pregnancy urine volumes can increase by 25%,[84] and by expressing DAP metabolites on a creatinine basis we were able to account for this. Another advantage of our study is that it is a large population-based prospective cohort study, which comprises a broad range of contextual information. Therefore, we were able to investigate many potential sources of OP pesticide exposure and were able to account for various confounding variables.

We should, however, be cautious when generalizing the factors associated with increased OP pesticide exposure in pregnant women in the Netherlands. The Generation R Study is representative of an urban population with varying ethnicities, SES, and educational level and not generalizable to semi-urban and rural areas in the Netherlands where the source of OP pesticide exposure could be different.

Overall, this study strengthens the hypothesis that dietary intake plays an important role in prenatal OP pesticide exposure among women living in an urban environment. Previous epidemiological studies have suggested that prenatal exposure to OP pesticides is associated with adverse neurodevelopmental and birth outcomes among children, but overall were inconclusive.[24-26] Considerably higher DAP metabolite concentrations were found in the Generation R population compared to other cohorts. Further research will be undertaken to investigate the possible health effects of this relatively high level of OP pesticide exposure in the offspring of the Generation R study, an urban population-based cohort.

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Table S1 Multivariable determinants of dialkyl phosphates metabolite concentrations on a creatinine basis (nmol/g creatinine) across pregnancy among 784 women participating in the Generation R cohort, with replacement of the values below the limit of detection with the LOD/√2 substitution method.

	Total dialkyl phosphates [a]		Dimethyl alkyl phosphates [b]		Diethyl alkyl phosphates [c]	
Determinants	B (95%CI)	P	B (95%CI)	P	B (95%CI)	P
Age in years	0.004 (0.001 to 0.008)	0.047*	0.003 (-0.001 to 0.007)	0.099	0.006 (0.001 to 0.010)	0.019*
Marital status						
Married/ partner	0.098 (0.045 to 0.151)	<0.001*	0.106 (0.051 to 0.161)	<0.001*	-	
No partner	ref		ref		-	
BMI						
<18.5	0.036 (-0.063 to 0.136)	0.475	0.035 (-0.068 to 0.139)	0.504	0.017 (-0.107 to 0.140)	0.793
18.5-<25	ref		ref		ref	
25-<30	-0.042 (-0.077 to -0.005)	0.024*	-0.039 (-0.076 to -0.002)	0.041*	-0.055 (-0.099 to -0.011)	0.014*
≥30	-0.092 (-0.149 to -0.036)	0.001*	-0.087 (-0.146 to -0.029)	0.003*	-0.161 (-0.229 to -0.093)	<0.001*
Parity						
0	0.085 (0.033 to 0.137)	0.001*	0.073 (0.020 to 0.127)	0.008*	0.120 (0.057 to 0.182)	<0.001*
1	0.059 (0.006 to 0.113)	0.030*	0.054 (-0.002 to 0.110)	0.058	0.077 (0.011 to 0.143)	0.022*
≥2	ref		ref		ref	
Education						
high	0.061 (0.007 to 0.115)	0.027*	0.070 (0.014 to 0.125)	0.014*	-	
medium	0.033 (-0.016 to 0.082)	0.190	0.041 (-0.009 to 0.092)	0.110	-	
low	ref		ref		-	
Income						
high	-		-		0.111 (0.039 to 0.183)	0.003*
medium	-		-		0.072 (-0.002 to 0.146)	0.058
low	-		-		ref	
Ethnicity						
Non-western	0.043 (0.005 to 0.082)	0.028*	0.060 (0.021 to 0.100)	0.003*	-	
Other western	-0.021 (-0.068 to 0.025)	0.363	-0.018 (-0.066 to 0.031)	0.486	-	
Dutch	ref		ref		ref	

Continue

Continued

	Total dialkyl phosphates [a]		Dimethyl alkyl phosphates [b]		Diethyl alkyl phosphates [c]	
Determinants	B (95%CI)	P	B (95%CI)	P	B (95%CI)	P
Smoking						
no smoking during pregnancy	0.091 (0.043 to 0.140)	<0.001*	0.083 (0.033 to 0.133)	0.001*	0.138 (0.079 to 0.197)	<0.001*
Until pregnancy recognized	0.102 (0.038 to 0.167)	0.002*	0.094 (0.028 to 0.161)	0.006*	0.141 (0.064 to 0.217)	<0.001*
continued during pregnancy	ref		ref		ref	
Work with pesticides						
Do not know	0.251 (0.015 to 0.487)	0.037*	-		0.391 (0.101 to 0.682)	0.008*
No	0.181 (-0.027 to 0.389)	0.089	-		0.275 (0.020 to 0.531)	0.035*
Yes	ref		-		ref	
Season						
Autumn	-0.046 (-0.080 to -0.012)	0.008*	-0.064 (-0.101 to -0.028)	<0.001*	0.043 (-0.001 to 0.086)	0.054
Winter	-0.033 (-0.069 to 0.003)	0.068	-0.047 (-0.085 to -0.009)	0.016*	0.055 (0.013 to 0.097)	0.010*
Spring	-0.020 (-0.051 to 0.011)	0.207	-0.022 (-0.055 to 0.011)	0.184	ref	
Summer	ref		ref		0.018 (-0.020 to 0.057)	0.353
Dog ownership						
No due to allergy	0.063 (-0.009 to 0.135)	0.085	-		0.060 (-0.029 to 0.148)	0.188
No	0.063 (0.001 to 0.125)	0.048*	-		0.078 (0.004 to 0.152)	0.049*
Yes	ref		-		ref	

a. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP, and DMP.
b. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP, and DMP.
c. Diethyl alkyl phosphates is the sum of DEDTP, DETP, and DEP.
* $p<0.05$.

Table S2. Associations [a] between the intake of food groups per 100g/d and dialkyl phosphates metabolite concentrations on a creatinine basis (nmol/g creatinine) across pregnancy among 610 pregnant women participating in the Generation R cohort, with replacement of the values below the limit of detection with the LOD/√2 substitution method.

	Total dialkyl phosphates [b]		Dimethyl alkyl phosphates [c]		Diethyl alkyl phosphates [d]	
Food intake	B (95%CI)	P	B (95%CI)	P	B (95%CI)	P
Per 100g/d						
Vegetables	0.001 (-0.028 to 0.030)	0.945	-0.008 (-0.037 to 0.022)	0.620	0.026 (-0.010 to 0.062)	0.151
Fruits	0.030 (0.016 to 0.045)	<0.001*	0.030 (0.015 to 0.046)	<0.001*	0.031 (0.013 to 0.049)	0.001*
Nuts	0.078 (-0.114 to 0.269)	0.427	0.090 (-0.110 to 0.289)	0.377	0.086 (-0.153 to 0.324)	0.481
Dairy	-0.003 (-0.011 to 0.005)	0.432	-0.003 (-0.011 to 0.005)	0.395	-0.002 (-0.012 to 0.007)	0.653
Fish	0.121 (-0.002 to 0.244)	0.054	0.114 (-0.015 to 0.242)	0.083	0.065 (-0.087 to 0.218)	0.405
Grain	-0.006 (-0.037 to 0.024)	0.686	-0.016 (-0.048 to 0.016)	0.326	0.018 (-0.019 to 0.056)	0.340
Meat	-0.031 (-0.078 to 0.017)	0.206	-0.029 (-0.079 to 0.021)	0.252	-0.035 (-0.094 to 0.025)	0.252

a. Adjusted for energy intake, maternal age, BMI categories (>18,5, 18,5-25, 25-30, 30+), parity categories (0,1,2+), ethnicity categories (Dutch, other-western, non-western), education categories (low, intermediate, high), household income categories (<1200 per month, 1200–2000 per month > 2000 per month), marital status, smoking categories (no smoking during pregnancy, smoked until pregnancy was known, smoked during pregnancy), and season of urine collection (fall, winter, spring, summer).

b. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP, and DMP.

c. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP, and DMP.

d. Diethyl alkyl phosphates is the sum of DEDTP, DETP, and DEP.

* $p<0.05$.

Table S3. Associations [a] between the intake of fruit types and dialkyl phosphates metabolite concentrations on a creatinine basis (nmol/g creatinine) across pregnancy among 610 pregnant women participating in the Generation R cohort, with replacement of the values below the limit of detection with the LOD/√2 substitution method.

	Total dialkyl phosphates [b]		Dimethyl alkyl phosphates [c]		Diethyl alkyl phosphates [d]	
Fruit intake	B (95%CI)	P	B (95%CI)	P	B (95%CI)	P
Mandarin, per 100g/d	0.040 (-0.044 to 0.125)	0.351	0.045 (-0.044 to 0.134)	0.313	-0.017 (-0.122 to 0.088)	0.746
Orange/grapefruit, per100g/d	0.053 (0.012 to 0.093)	0.011*	0.057 (0.014 to 0.099)	0.009*	0.040 (-0.010 to 0.091)	0.118
Lemon/lime, yes	0.031 (-0.004 to 0.065)	0.080	0.028 (-0.008 to 0.064)	0.128	0.044 (0.001 to 0.087)	0.043*
Banana, per 100g/d	0.011 (-0.052 to 0.073)	0.735	0.012 (-0.053 to 0.078)	0.713	0.021 (-0.056 to 0.099)	0.592
Kiwi, yes	0.021 (-0.018 to 0.059)	0.295	0.004 (-0.036 to 0.044)	0.847	0.079 (0.032 to 0.126)	0.001*
Apple, per 100g/d	0.057 (0.015 to 0.099)	0.008*	0.065 (0.021 to 0.109)	0.004*	0.032 (-0.020 to 0.084)	0.239
Pear, yes	0.017 (-0.018 to 0.052)	0.331	0.011 (-0.027 to 0.048)	0.573	0.024 (-0.020 to 0.069)	0.282
Mango, yes	0.019 (-0.017 to 0.055)	0.295	0.014 (-0.024 to 0.052)	0.466	0.045 (-0.001 to 0.089)	0.052
Avocado, yes	-0.001 (-0.038 to 0.036)	0.956	-0.004 (-0.042 to 0.035)	0.842	-0.002 (-0.049 to 0.044)	0.919
Peach/nectarine, yes	0.017 (-0.018 to 0.052)	0.336	0.018 (-0.018 to 0.055)	0.326	0.026 (-0.017 to 0.069)	0.235
Apricot, yes	0.063 (0.018 to 0.108)	0.006*	0.064 (0.017 to 0.111)	0.007*	0.068 (0.012 to 0.124)	0.017*
Plum, yes	0.027 (-0.010 to 0.063)	0.150	0.025 (-0.013 to 0.063)	0.204	0.038 (-0.008 to 0.083)	0.104
Strawberry/raspberry, yes	0.008 (-0.029 to 0.045)	0.668	-0.003 (-0.041 to 0.036)	0.897	0.050 (0.004 to 0.096)	0.032*
Grape/cherry, yes	0.052 (0.015 to 0.088)	0.005*	0.054 (0.016 to 0.092)	0.006*	0.041 (-0.004 to 0.087)	0.074
Pineapple/melon, yes	0.015 (-0.021 to 0.051)	0.412	0.007 (-0.031 to 0.044)	0.724	0.053 (0.009 to 0.097)	0.019*
Canned fruit, yes	0.005 (-0.036 to 0.046)	0.810	0.003 (-0.040 to 0.046)	0.885	0.028 (-0.023 to 0.079)	0.287

a. Adjusted for energy intake, maternal age, BMI categories (>18,5, 18,5-25, 25-30, 30+), parity categories (0,1,2+), ethnicity categories (Dutch, other-western, non-western), education categories (low, intermediate, high), household income categories (<1200 per month, 1200–2000 per month > 2000 per month), marital status, smoking categories (no smoking during pregnancy, smoked until pregnancy was known, smoked during pregnancy), and season of urine collection (fall, winter, spring, summer).

b. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP, and DMP.

c. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP, and DMP.

d. Diethyl alkyl phosphates is the sum of DEDTP, DETP, and DEP.

* $p<0.05$.

Table S4. Results [a] of the multiple regression analyses presenting the associations between the intake of food groups per 100g/d and dialkyl phosphates metabolite concentrations on a creatinine basis (nmol/g creatinine) at < 18 weeks of gestation among 610 pregnant women participating in the Generation R cohort.

	Total dialkyl phosphates [b]		Dimethyl alkyl phosphates [c]		Diethyl alkyl phosphates [d]	
Food intake	B (95%CI)	P	B(95%CI)	P	B (95%CI)	P
per 100g/d						
Vegetables	-0.015 (-0.059 to 0.029)	0.511	-0.020 (-0.066 to 0.026)	0.389	0.003 (-0.050 to 0.055)	0.924
Fruits	0.033 (0.011 to 0.056)	0.004*	0.034 (0.010 to 0.058)	0.006*	0.033 (0.006 to 0.060)	0.017*
Nuts	0.038 (-0.256 to 0.331)	0.802	0.017 (-0.292 to 0.325)	0.916	0.287 (-0.061 to 0.635)	0.106
Dairy	0.000 (-0.012 to 0.011)	0.947	-0.003 (-0.016 to 0.009)	0.580	0.007 (-0.007 to 0.021)	0.310
Fish	0.101 (-0.087 to 0.289)	0.291	0.111 (-0.086 to 0.308)	0.268	-0.004 (-0.228 to 0.220)	0.971
Grain	-0.010 (-0.056 to 0.036)	0.677	-0.017 (-0.066 to 0.031)	0.491	0.008 (-0.047 to 0.063)	0.770
Meat	-0.058 (-0.132 to 0.015)	0.120	-0.053 (-0.130 to 0.024)	0.175	-0.068 (-0.156 to 0.020)	0.131

a. Adjusted for energy intake, maternal age, BMI categories (>18,5, 18,5-25, 25-30, 30+), parity categories (0,1,2+), ethnicity categories (Dutch, other-western, non-western), education categories (low, intermediate, high), IQ mother, household income categories (<1200 per month, 1200–2000 per month > 2000 per month), marital status, smoking categories (no smoking during pregnancy, smoked until pregnancy was known, smoked during pregnancy), and season of urine collection (fall, winter, spring, summer)

b. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP, and DMP.

c. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP, and DMP.

d. Diethyl alkyl phosphates is the sum of DEDTP, DETP, and DEP.

* $p<0.05$.

Table S5. Results [a] of the multiple regression analyses presenting the associations between the intake of fruit types and dialkyl phosphates metabolite concentrations on a creatinine basis (nmol/g creatinine) at < 18 weeks of gestation among 610 pregnant women participating in the Generation R cohort.

	Total dialkyl phosphates [b]		Dimethyl alkyl phosphates [c]		Diethyl alkyl phosphates [d]	
Fruit intake	B (95%CI)	P	B (95%CI)	P	B (95%CI)	P
Mandarin, per 100g/d	0.008 (-0.128 to 0.145)	0.907	-0.015 (-0.159 to 0.128)	0.832	0.038 (-0.125 to 0.219)	0.645
Orange/grapefruit, per 100g/d	0.065 (0.002 to 0.128)	0.043*	0.071 (0.005 to 0.137)	0.036*	0.049 (-0.026 to 0.124)	0.198
Lemon/lime, yes	0.060 (0.007 to 0.112)	0.027*	0.053 (-0.002 to 0.109)	0.060	0.072 (0.009 to 0.135)	0.025*
Banana, per 100g/d	0.015 (-0.082 to 0.113)	0.756	0.024 (-0.079 to 0.127)	0.649	-0.001 (-0.115 to 0.113)	0.990
Kiwi, yes	0.032 (-0.026 to 0.091)	0.280	0.014 (-0.048 to 0.075)	0.664	0.083 (0.013 to 0.153)	0.021*
Apple, per 100g/d	0.116 (0.051 to 0.180)	<0.001*	0.131 (0.063 to 0.198)	<0.001*	0.076 (-0.001 to 0.153)	0.051
Pear, yes	0.030 (-0.025 to 0.085)	0.287	0.019 (-0.039 to 0.077)	0.512	0.056 (-0.009 to 0.121)	0.091
Mango, yes	0.028 (-0.027 to 0.083)	0.314	0.020 (-0.038 to 0.078)	0.495	0.035 (-0.032 to 0.101)	0.307
Avocado, yes	0.011 (-0.046 to 0.068)	0.668	0.013 (-0.046 to 0.073)	0.663	-0.021 (-0.089 to 0.047)	0.550
Peach/nectarine, yes	0.036 (-0.023 to 0.094)	0.230	0.046 (-0.015 to 0.107)	0.141	-0.007 (-0.076 to 0.061)	0.830
Apricot, yes	0.142 (0.074 to 0.211)	<0.001*	0.157 (0.085 to 0.229)	<0.001*	0.056 (-0.026 to 0.139)	0.182
Plum, yes	0.044 (-0.012 to 0.100)	0.122	0.043 (-0.016 to 0.102)	0.156	0.034 (-0.033 to 0.101)	0.317
Strawberry/raspberry, yes	0.013 (-0.047 to 0.072)	0.670	0.007 (-0.055 to 0.070)	0.816	0.038 (-0.033 to 0.110)	0.295
Grape/cherries, yes	0.027 (-0.030 to 0.083)	0.354	0.035 (-0.025 to 0.094)	0.254	-0.006 (-0.074 to 0.062)	0.858
Pineapple/melon, yes	0.024 (-0.032 to 0.081)	0.399	0.015 (-0.045 to 0.074)	0.626	0.063 (-0.004 to 0.129)	0.065
Canned fruit, yes	0.024 (-0.039 to 0.088)	0.453	0.021 (-0.046 to 0.088)	0.531	0.028 (-0.049 to 0.104)	0.478

a. Adjusted for energy intake, maternal age, BMI categories (>18,5, 18,5-25, 25-30, 30+), parity categories (0,1,2+), ethnicity categories (Dutch, other-western, non-western), education categories (low, intermediate, high), IQ mother, household income categories (<1200 per month, 1200–2000 per month > 2000 per month), marital status, smoking categories (no smoking during pregnancy, smoked until pregnancy was known, smoked during pregnancy), and season of urine collection (fall, winter, spring, summer).

b. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP, and DMP.

c. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP, and DMP.

d. Diethyl alkyl phosphates is the sum of DEDTP, DETP, and DEP.

* $p<0.05$. t

Table S6. Associations [a] between mutually adjusted food groups per 100g/d and dialkyl phosphates metabolite concentrations on a creatinine basis (nmol/g creatinine) across pregnancy among 610 pregnant women participating in the Generation R cohort

	Total dialkyl phosphates [b]		Dimethyl alkyl phosphates [c]		Diethyl alkyl phosphates [d]	
Food intake	B (95%CI)	P	B (95%CI)	P	B (95%CI)	P
Per 100g/d						
Vegetables	-0.014 (-0.043 to 0.016)	0.369	-0.021 (-0.052 to 0.009)	0.182	0.011 (-0.026 to 0.049)	0.544
Fruits	0.031 (0.016 to 0.046)	<0.001*	0.031 (0.015 to 0.046)	<0.001*	0.030 (0.012 to 0.049)	0.002*
Nuts	0.051 (-0.146 to 0.249)	0.611	0.068 (-0.138 to 0.274)	0.519	0.046 (-0.202 to 0.293)	0.716
Dairy	-0.005 (-0.013 to 0.003)	0.211	-0.006 (-0.014 to 0.002)	0.166	-0.003 (-0.013 to 0.007)	0.538
Fish	0.119 (-0.006 to 0.224)	0.062	0.120 (-0.009 to 0.250)	0.069	0.035 (-0.121 to 0.190)	0.663
Grain	-0.003 (-0.034 to 0.028)	0.860	-0.012 (-0.044 to 0.021)	0.483	0.021 (-0.018 to 0.060)	0.294
Meat	-0.022 (-0.072 to 0.027)	0.380	-0.019 (-0.071 to 0.032)	0.466	-0.026 (-0.089 to 0.036)	0.408

a. Adjusted for energy intake, maternal age, BMI categories (>18,5, 18,5-25, 25-30, 30+), parity categories (0,1,2+), ethnicity categories (Dutch, other-western, non-western), education categories (low, intermediate, high), household income categories (<1200 per month, 1200–2000 per month > 2000 per month), marital status, smoking categories (no smoking during pregnancy, smoked until pregnancy was known, smoked during pregnancy), and season of urine collection (fall, winter, spring, summer).

b. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP, and DMP.

c. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP, and DMP.

d. Diethyl alkyl phosphates is the sum of DEDTP, DETP, and DEP.

* $p<0.05$.

Table S7. Associations [a] between the intake of fruit per category in grams and dialkyl phosphates metabolite concentrations on a creatinine basis (nmol/g creatinine) across pregnancy among 610 pregnant women participating in the Generation R cohort

	Total dialkyl phosphates [b]		Dimethyl alkyl phosphates [c]		Diethyl alkyl phosphates [d]	
Fruit intake per category in grams	B (95%CI)	P	B (95%CI)	P	B (95%CI)	P
<50 g	ref		ref		ref	
50 – 99 g	0.010 (-0.051 to 0.070)	0.753	0.010 (-0.053 to 0.074)	0.753	0.018 (-0.058 to 0.093)	0.649
100 – 149 g	0.066 (0.014 to 0.119)	0.014*	0.063 (0.008 to 0.118)	0.026*	0.087 (0.021 to 0.154)	0.010*
150 – 199 g	0.092 (0.037 to 0.146)	0.001*	0.087 (0.030 to 0.144)	0.003*	0.106 (0.038 to 0.174)	0.002*
≥200 g	0.109 (0.054 to 0.164)	<0.001*	0.106 (0.048 to 0.164)	<0.001*	0.112 (0.043 to 0.182)	0.001*

a. Adjusted for energy intake, maternal age, BMI categories (>18,5, 18,5-25, 25-30, 30+), parity categories (0,1,2+), ethnicity categories (Dutch, other-western, non-western), education categories (low, intermediate, high), household income categories (<1200 per month, 1200–2000 per month > 2000 per month), marital status, smoking categories (no smoking during pregnancy, smoked until pregnancy was known, smoked during pregnancy), and season of urine collection (fall, winter, spring, summer).
b. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP, and DMP.
c. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP, and DMP.
d. Diethyl alkyl phosphates is the sum of DEDTP, DETP, and DEP.
* $p<0.05$.

Table S8. Multivariable determinants of dialkyl phosphates metabolite concentrations in nmol/L adjusted for creatinine across pregnancy among 784 women participating in the Generation R cohort

	Total dialkyl phosphates [a,d]		Dimethyl alkyl phosphates [b,d]		Diethyl alkyl phosphates [c,d]	
Determinants	B (95%CI)	P	B (95%CI)	P	B (95%CI)	P
Age in years	0.003 (-0.002 to 0.007)	0.229	0.002 (-0.002 to 0.006)	0.406	0.005 (-0.001 to 0.010)	0.060
Marital status						
Married/ partner	0.080 (0.022 to 0.137)	0.006*	0.087 (0.028 to 0.146)	0.004*	-	
No partner	ref		ref		-	
BMI						
<18.5	0.030 (-0.076 to 0.135)	0.580	0.026 (-0.083 to 0.136)	0.637	0.011 (-0.118 to 0.140)	0.864
18.5-<25	ref		ref		ref	
25-<30	-0.034 (-0.072 to 0.004)	0.082	-0.031 (-0.071 to 0.008)	0.120	-0.051 (-0.097 to -0.005)	0.031*
≥30	-0.057 (-0.117 to 0.003)	0.063	-0.050 (-0.112 to 0.012)	0.114	-0.133 (-0.205 to -0.061)	<0.001*
Parity						
0	0.051 (-0.004 to 0.105)	0.070	0.038 (-0.018 to 0.095)	0.184	0.089 (0.023 to 0.154)	0.008*
1	0.038 (-0.018 to 0.095)	0.185	0.033 (-0.026 to 0.092)	0.270	0.056 (-0.012 to 0.125)	0.108
≥2	ref		ref		ref	
Education						
high	0.064 (0.007 to 0.122)	0.028*	0.069 (0.011 to 0.127)	0.020*	-	
medium	0.024 (-0.028 to 0.075)	0.374	0.030 (-0.024 to 0.084)	0.277	-	
low	ref		ref		-	
Income						
high	-		-		0.103 (0.026 to 0.180)	0.010*
medium	-		-		0.055 (-0.025 to 0.136)	0.177
low	-		-		ref	
Ethnicity						
Non-western	0.042 (0.001 to 0.082)	0.047*	0.057 (0.016 to 0.099)	0.007*	-	
Other western	-0.016 (-0.065 to 0.033)	0.516	-0.013 (-0.064 to 0.038)	0.615	-	
Dutch	ref		ref		ref	
Smoking						
no smoking during pregnancy	0.081 (0.029 to 0.134)	0.002*	0.070 (0.017 to 0.124)	0.010*	0.128 (0.067 to 0.190)	<0.001*
Until pregnancy recognized	0.087 (0.018 to 0.156)	0.014*	0.076 (0.005 to 0.146)	0.035*	0.126 (0.044 to 0.208)	0.003*
continued during pregnancy	ref		ref		ref	

Continue

Continued

	Total dialkyl phosphates [a,d]		Dimethyl alkyl phosphates [b,d]		Diethyl alkyl phosphates [c,d]	
Determinants	B (95%CI)	P	B (95%CI)	P	B (95%CI)	P
Work with pesticides						
Do not know	0.260 (0.010 to 0.510)	0.041*	-		0.404 (0.101 to 0.707)	0.009*
No	0.167 (-0.054 to 0.387)	0.138	-		0.270 (0.002 to 0.539)	0.048*
Yes	ref		-		ref	
Season						
Autumn	-0.064 (-0.101 to -0.026)	0.001*	-0.082 (-0.121 to -0.042)	<0.001*	0.029 (-0.017 to 0.074)	0.209
Winter	-0.044 (-0.084 to -0.005)	0.026*	-0.057 (-0.098 to -0.016)	0.006*	0.048 (0.004 to 0.092)	0.032*
Spring	-0.025 (-0.059 to 0.009)	0.155	-0.027 (-0.063 to 0.009)	0.143	ref	
Summer	ref		ref		0.022 (-0.018 to 0.062)	0.290
Dog ownership						
No due to allergy	0.039 (-0.038 to 0.115)	0.320	-		0.036 (-0.056 to 0.128)	0.443
No	0.043 (-0.023 to 0.108)	0.202	-		0.059 (-0.018 to 0.137)	0.135
Yes	ref		-		ref	

a. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP, and DMP.
b. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP, and DMP.
c. Diethyl alkyl phosphates is the sum of DEDTP, DETP, and DEP.
d. Adjusted for creatinine.
* $p<0.05$.

Table S9. Associations [a] between the intake of food groups per 100g/d and dialkyl phosphates metabolite concentrations in nmol/L adjusted for creatinine across pregnancy among 610 pregnant women participating in the Generation R cohort

	Total dialkyl phosphates [b]		Dimethyl alkyl phosphates [c]		Diethyl alkyl phosphates [d]	
Food intake	B (95%CI)	P	B (95%CI)	P	B (95%CI)	P
Per 100g/d						
Vegetables	-0.020 (-0.050 to 0.011)	0.204	-0.029 (-0.061 to 0.003)	0.077	0.006 (-0.031 to 0.044)	0.743
Fruits	0.024 (0.009 to 0.040)	0.002*	0.024 (0.008 to 0.040)	0.004*	0.025 (0.061 to 0.044)	0.010*
Nuts	0.041 (-0.166 to 0.240)	0.719	0.049 (-0.162 to 0.261)	0.649	0.044 (-0.204 to 0.293)	0.726
Dairy	-0.005 (-0.013 to 0.004)	0.277	-0.005 (-0.014 to 0.003)	0.248	-0.003 (-0.013 to 0.007)	0.507
Fish	0.163 (0.032 to 0.293)	0.015*	0.155 (0.019 to 0.291)	0.026*	0.106 (-0.054 to 0.265)	0.194
Grain	0.001 (-0.032 to 0.032)	0.999	-0.010 (-0.043 to 0.024)	0.575	0.025 (-0.015 to 0.064)	0.219
Meat	-0.003(-0.053 to 0.048)	0.920	-0.001 (-0.053 to 0.052)	0.985	-0.007 (-0.069 to 0.055)	0.826

a. Adjusted for creatinine, energy intake, maternal age, BMI categories (>18,5, 18,5-25, 25-30, 30+), parity categories (0,1,2+), ethnicity categories (Dutch, other-western, non-western), education categories (low, intermediate, high), household income categories (<1200 per month, 1200–2000 per month > 2000 per month), marital status, smoking categories (no smoking during pregnancy, smoked until pregnancy was known, smoked during pregnancy), and season of urine collection (fall, winter, spring, summer).

b. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP, and DMP.

c. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP, and DMP.

d. Diethyl alkyl phosphates is the sum of DEDTP, DETP, and DEP.

* $p<0.05$.

Table S10. Associations [a] between the intake of fruit types and dialkyl phosphates metabolite concentrations in nmol/L adjusted for creatinine across pregnancy among 610 pregnant women participating in the Generation R cohort

	Total dialkyl phosphates [b]		Dimethyl alkyl phosphates [c]		Diethyl alkyl phosphates [d]	
Fruit intake	B (95%CI)	P	B (95%CI)	P	B (95%CI)	P
Mandarin, per 100g/d	0.017 (-0.073 to 0.107)	0.718	0.021 (-0.073 to 0.115)	0.664	-0.040 (-0.150 to 0.070)	0.474
Orange/grapefruit, per100g/d	0.042 (-0.001 to 0.086)	0.052	0.046 (0.015 to 0.091)	0.043*	0.031 (-0.022 to 0.084)	0.252
Lemon/lime, yes	0.019 (-0.017 to 0.056)	0.300	0.016 (-0.022 to 0.054)	0.402	0.033 (-0.012 to 0.077)	0.146
Banana, per 100g/d	-0.004 (-0.070 to 0.063)	0.912	-0.003 (-0.072 to 0.067)	0.942	0.008 (-0.072 to 0.088)	0.847
Kiwi, yes	0.007 (-0.034 to 0.048)	0.731	-0.010 (-0.052 to 0.033)	0.656	0.066 (0.017 to 0.116)	0.009*
Apple, per 100g/d	0.031 (-0.013 to 0.076)	0.170	0.039 (-0.007 to 0.086)	0.099	0.006 (-0.049 to 0.061)	0.824
Pear, yes	-0.003 (-0.041 to 0.035)	0.866	-0.008 (-0.048 to 0.032)	0.699	0.006 (-0.041 to 0.052)	0.810
Mango, yes	0.005 (-0.034 to 0.043)	0.809	-0.001 (-0.040 to 0.039)	0.978	0.029 (-0.017 to 0.076)	0.219
Avocado, yes	-0.001 (-0.040 to 0.039)	0.985	-0.003 (-0.044 to 0.038)	0.880	-0.002 (-0.050 to 0.046)	0.922
Peach/nectarine, yes	0.038 (0.001 to 0.075)	0.046*	0.039 (0.001 to 0.077)	0.049*	0.046 (0.001 to 0.090)	0.046*
Apricot, yes	0.062 (0.014 to 0.109)	0.011*	0.063 (0.013 to 0.112)	0.014*	0.067 (0.009 to 0.125)	0.025*
Plum, yes	0.027 (-0.011 to 0.066)	0.165	0.025 (-0.015 to 0.066)	0.217	0.038 (-0.010 to 0.085)	0.117
Strawberry/raspberry, yes	0.002 (-0.037 to 0.042)	0.903	-0.008 (-0.049 to 0.033)	0.700	0.044 (-0.004 to 0.091)	0.073
Grape/cherry, yes	0.055 (0.016 to 0.093)	0.006*	0.057 (0.016 to 0.097)	0.006*	0.044 (-0.003 to 0.092)	0.067
Pineapple/melon, yes	0.017 (-0.02- to 0.055)	0.365	0.009 (-0.030 to 0.049)	0.644	0.055 (0.009 to 0.101)	0.019*
Canned fruit, yes	0.014 (-0.030 to 0.057)	0.538	0.012 (-0.033 to 0.058)	0.598	0.036 (-0.017 to 0.089)	0.187

a. Adjusted for creatinine, energy intake, maternal age, BMI categories (>18,5, 18,5-25, 25-30, 30+), parity categories (0,1,2+), ethnicity categories (Dutch, other-western, non-western), education categories (low, intermediate, high), household income categories (<1200 per month, 1200–2000 per month > 2000 per month), marital status, smoking categories (no smoking during pregnancy, smoked until pregnancy was known, smoked during pregnancy), and season of urine collection (fall, winter, spring, summer.

b. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP, and DMP.

c. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP, and DMP.

d. Diethyl alkyl phosphates is the sum of DEDTP, DETP, and DEP.

* $p<0.05$.

Table S11. Associations [a] between the intake of food groups per 100g/d and dialkyl phosphates metabolite concentrations in nmol/L adjusted for creatinine at < 18 weeks of gestation among 610 pregnant women participating in the Generation R cohort

	Total dialkyl phosphates [b]		Dimethyl alkyl phosphates [c]		Diethyl alkyl phosphates [d]	
Food intake	B (95%CI)	P	B (95%CI)	P	B (95%CI)	P
Per 100g/d						
Vegetables	-0.033 (-0.081 to 0.014)	0.165	-0.039 (-0.089 to 0.011)	0.123	-0.015 (-0.070 to 0.039)	0.578
Fruits	0.034 (0.009 to 0.058)	0.007*	0.034 (0.008 to 0.060)	0.010*	0.033 (0.006 to 0.061)	0.019*
Nuts	0.034 (-0.282 to 0.349)	0.835	0.013 (-0.319 to 0.345)	0.938	0.281 (-0.079 to 0.641)	0.126
Dairy	-0.003 (-0.016 to 0.009)	0.613	-0.006 (-0.020 to 0.007)	0.346	0.005 (-0.010 to 0.019)	0.536
Fish	0.177 (-0.025 to 0.379)	0.085	0.188 (-0.025 to 0.400)	0.083	0.071 (-0.160 to 0.303)	0.547
Grain	-0.022 (-0.072 to 0.028)	0.390	-0.029 (-0.081 to 0.023)	0.275	-0.003 (-0.060 to 0.054)	0.913
Meat	-0.033 (-0.112 to 0.046)	0.418	-0.027 (-0.110 to 0.055)	0.517	-0.043 (-0.134 to 0.048)	0.358

a. Adjusted for creatinine, energy intake, maternal age, BMI categories (>18,5, 18,5-25, 25-30, 30+), parity categories (0,1,2+), ethnicity categories (Dutch, other-western, non-western), education categories (low, intermediate, high), household income categories (<1200 per month, 1200–2000 per month > 2000 per month), marital status, smoking categories (no smoking during pregnancy, smoked until pregnancy was known, smoked during pregnancy), season of urine collection (fall, winter, spring, summer).

b. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP, and DMP.

c. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP, and DMP.

d. Diethyl alkyl phosphates is the sum of DEDTP, DETP, and DEP.

* $p<0.05$.

Table S12. Associations [a] between the intake of fruit types and dialkyl phosphates metabolite concentrations in nmol/L adjusted for creatinine at < 18 weeks of gestation among 610 pregnant women participating in the Generation R cohort

	Total dialkyl phosphates [b]		Dimethyl alkyl phosphates [c]		Diethyl alkyl phosphates [d]	
Fruit intake	B (95%CI)	P	B (95%CI)	P	B (95%CI)	P
Mandarin, per 100g/d	0.006 (-0.141 to 0.153)	0.937	-0.018 (-0.173 to 0.136)	0.815	0.039 (-0.130 to 0.207)	0.653
Orange/grapefruit, per 100g/d	0.077 (0.010 to 0.145)	0.025*	0.083 (0.012 to 0.154)	0.023*	0.062 (-0.016 to 0.139)	0.118
Lemon/lime, yes	0.051 (-0.006 to 0.108)	0.078	0.045 (-0.015 to 0.104)	0.144	0.064 (-0.001 to 0.129)	0.054
Banana, per 100g/d	-0.020 (-0.125 to 0.085)	0.705	-0.012 (-0.123 to 0.099)	0.831	-0.035 (-0.153 to 0.083)	0.563
Kiwi, yes	0.021 (-0.042 to 0.084)	0.514	0.002 (-0.064 to 0.069)	0.947	0.072 (-0.001 to 0.144)	0.053
Apple, per 100g/d	0.097 (0.027 to 0.166)	0.006*	0.111 (0.038 to 0.184)	0.003*	0.058 (-0.022 to 0.138)	0.154
Pear, yes	0.014 (-0.046 to 0.073)	0.646	0.003 (-0.059 to 0.066)	0.914	0.039 (-0.028 to 0.107)	0.253
Mango, yes	0.027 (-0.032 to 0.086)	0.368	0.019 (-0.043 to 0.081)	0.551	0.033 (-0.035 to 0.102)	0.341
Avocado, yes	0.017 (-0.044 to 0.078)	0.546	0.019 (-0.045 to 0.084)	0.561	-0.016 (-0.086 to 0.054)	0.660
Peach/nectarine, yes	0.080 (0.018 to 0.142)	0.012*	0.091 (0.025 to 0.156)	0.007*	0.036 (-0.035 to 0.106)	0.322
Apricot, yes	0.152 (0.079 to 0.226)	<0.001*	0.167 (0.089 to 0.244)	<0.001*	0.066 (-0.020 to 0.151)	0.131
Plum, yes	0.059 (-0.001 to 0.120)	0.055	0.058 (-0.006 to 0.121)	0.075	0.048 (-0.021 to 0.117)	0.175
Strawberry/raspberry, yes	0.023 (-0.041 to 0.087)	0.713	0.018 (-0.049 to 0.085)	0.601	0.048 (-0.026 to 0.122)	0.205
Grape/cherry, yes	0.028 (-0.033 to 0.089)	0.909	0.036 (-0.028 to 0.100)	0.269	-0.005 (-0.075 to 0.065)	0.883
Pineapple/melon, yes	0.023 (-0.038 to 0.083)	0.466	0.013 (-0.051 to 0.077)	0.687	0.060 (-0.009 to 0.130)	0.086
Canned fruit, yes	0.038 (-0.030 to 0.107)	0.269	0.036 (-0.036 to 0.107)	0.332	0.041 (-0.038 to 0.119)	0.307

a. Adjusted for creatinine, energy intake, maternal age, BMI categories (>18,5, 18,5-25, 25-30, 30+), parity categories (0,1,2+), ethnicity categories (Dutch, other-western, non-western), education categories (low, intermediate, high), household income categories (<1200 per month, 1200–2000 per month > 2000 per month), marital status, smoking categories (no smoking during pregnancy, smoked until pregnancy was known, smoked during pregnancy), and season of urine collection (fall, winter, spring, summer).
b. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP, and DMP.
c. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP, and DMP.
d. Diethyl alkyl phosphates is the sum of DEDTP, DETP, and DEP.
* $p<0.05$

Table S13. Multivariable determinants of dialkyl phosphates metabolite concentrations on a creatinine basis (nmol/g creatinine) across pregnancy among women participating in the Generation R cohort with complete data

	Total dialkyl phosphates [a,d]		Dimethyl alkyl phosphates [b,e]		Diethyl alkyl phosphates [c,f]	
Determinants	B (95%CI)	P	B (95%CI)	P	B (95%CI)	P
Age in years	0.005 (0.001 to 0.009)	0.046*	0.003 (-0.001 to 0.007)	0.195	0.007 (0.001 to 0.013)	0.019*
Marital status						
Married/ partner	0.114 (0.053 to 0.174)	<0.001*	0.115 (0.059 to 0.172)	<0.001*	-	
No partner	ref		ref		-	
BMI						
<18.5	0.062 (-0.047 to 0.172)	0.265	0.040 (-0.064 to 0.143)	0.450	0.070 (-0.078 to 0.218)	0.352
18.5-<25	ref		ref		ref	
25-<30	-0.034 (-0.075 to 0.006)	0.099	-0.025 (-0.065 to 0.014)	0.212	-0.061 (-0.114 to -0.007)	0.027*
≥30	-0.089 (-0.155 to -0.023)	0.009*	-0.079 (-0.142 to -0.017)	0.013*	-0.170 (-0.253 to -0.088)	<0.001*
Parity						
0	0.087 (0.022 to 0.151)	0.009*	0.075 (0.015 to 0.135)	0.014*	0.093 (0.010 to 0.176)	0.029*
1	0.074 (0.008 to 0.140)	0.028*	0.059 (-0.003 to 0.121)	0.061	0.053 (-0.033 to 0.139)	0.229
≥2	ref		ref		ref	
Education						
high	0.031 (-0.031 to 0.094)	0.324	0.061 (0.036 to 0.118)	0.037*	-	
medium	0.013 (-0.045 to 0.071)	0.662	0.029 (-0.024 to 0.082)	0.287	-	
low	ref		ref		-	
Income						
high	-		-		0.120 (0.043 to 0.197)	0.002*
medium	-		-		0.069 (-0.017 to 0.156)	0.117
low	-		-		ref	
Ethnicity						
Non-western	0.048 (0.003 to 0.093)	0.035*	0.057 (0.015 to 0.099)	0.008*	-	
Other western	-0.030 (-0.080 to 0.021)	0.244	-0.015 (-0.065 to 0.034)	0.542	-	
Dutch	ref		ref		ref	
Smoking						
no smoking during pregnancy	0.069 (0.012 to 0.127)	0.018*	0.077 (0.026 to 0.128)	0.003*	0.105 (0.031 to 0.178)	0.006*
Until pregnancy recognized	0.095 (0.019 to 0.171)	0.015*	0.091 (0.020 to 0.162)	0.012*	0.101 (0.002 to 0.200)	0.046*
continued during pregnancy	ref		ref		ref	

Continue

Continued

	Total dialkyl phosphates [a,d]		Dimethyl alkyl phosphates [b,e]		Diethyl alkyl phosphates [c,f]	
Determinants	B (95%CI)	P	B (95%CI)	P	B (95%CI)	P
Work with pesticides						
Do not know	0.213 (-0.024 to 0.450)	0.078	-		0.320 (0.014 to 0.625)	0.041*
No	0.176 (-0.026 to 0.377)	0.087	-		0.265 (0.005 to 0.526)	0.046*
Yes	ref		-		ref	
Season						
Autumn	-0.044 (-0.084 to -0.005)	0.027*	-0.063 (-0.101 to -0.025)	0.001*	0.034 (-0.018 to 0.086)	0.204
Winter	-0.050 (-0.091 to -0.008)	0.018*	-0.055 (-0.095 to -0.015)	0.008*	0.043 (-0.009 to 0.094)	0.104
Spring	-0.009 (-0.044 to 0.027)	0.624	-0.019 (-0.053 to 0.015)	0.281	ref	
Summer	ref		ref		0.013 (-0.033 to 0.058)	0.585
Dog ownership						
No due to allergy	0.112 (0.028 to 0.195)	0.009*	-		0.102 (-0.006 to 0.210)	0.064
No	0.101 (0.031 to 0.172)	0.005*	-		0.104 (0.014 to 0.194)	0.023*
Yes	ref		-		ref	

a. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP, and DMP.
b. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP, and DMP.
c. Diethyl alkyl phosphates is the sum of DEDTP, DETP, and DEP.
d. N=592.
e. N=702.
f. N=576.
* $p<0.05$.

Table S14. Associations [a] between the intake of food groups per 100g/d and dialkyl phosphates metabolite concentrations on a creatinine basis (nmol/g creatinine) across pregnancy among 497 pregnant women participating in the Generation R cohort with complete data

	Total dialkyl phosphates [b]		Dimethyl alkyl phosphates [c]		Diethyl alkyl phosphates [d]	
Food intake	B (95%CI)	P	B (95%CI)	P	B (95%CI)	P
Per 100g/d						
Vegetables	-0.001 (-0.032 to 0.031)	0.984	-0.011 (-0.043 to 0.022)	0.512	0.030 (-0.011 to 0.070)	0.149
Fruits	0.027 (0.011 to 0.043)	0.001*	0.027 (0.011 to 0.043)	0.001*	0.023 (0.003 to 0.044)	0.023*
Nuts	0.088 (-0.126 to 0.302)	0.420	0.114 (-0.109 to 0.337)	0.317	0.124 (-0.152 to 0.401)	0.378
Dairy	-0.004 (-0.012 to 0.004)	0.343	-0.003 (-0.011 to 0.006)	0.500	-0.007 (-0.018 to 0.003)	0.169
Fish	0.101 (-0.048 to 0.250)	0.183	0.082 (-0.071 to 0.234)	0.293	0.130 (-0.062 to 0.322)	0.184
Grain	-0.015 (-0.050 to 0.019)	0.383	-0.031 (-0.066 to 0.005)	0.088	0.022 (-0.022 to 0.066)	0.324
Meat	-0.019 (-0.071 to 0.033)	0.468	-0.015 (-0.070 to 0.039)	0.575	-0.029 (-0.096 to 0.039)	0.405

a. Adjusted for energy intake, maternal age, BMI categories (>18,5, 18,5-25, 25-30, 30+), parity categories (0,1,2+), ethnicity categories (Dutch, other-western, non-western), education categories (low, intermediate, high), household income categories (<1200 per month, 1200–2000 per month > 2000 per month), marital status, smoking categories (no smoking during pregnancy, smoked until pregnancy was known, smoked during pregnancy), and season of urine collection (fall, winter, spring, summer).

b. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP, and DMP.

c. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP, and DMP.

d. Diethyl alkyl phosphates is the sum of DEDTP, DETP, and DEP.

* $p<0.05$.

Table S15. Associations [a] between the intake of fruit types and dialkyl phosphates metabolite concentrations on a creatinine basis (nmol/g creatinine) across pregnancy among 497 pregnant women participating in the Generation R cohort with complete data

	Total dialkyl phosphates [b]		Dimethyl alkyl phosphates [c]		Diethyl alkyl phosphates [d]	
Fruit intake	B (95%CI)	P	B (95%CI)	P	B (95%CI)	P
Mandarin, per 100g/d	0.026 (-0.063 to 0.116)	0.567	0.038 (-0.055 to 0.131)	0.419	-0.032 (-0.147 to 0.084)	0.591
Orange/grapefruit, per 100g/d	0.046 (0.002 to 0.090)	0.042*	0.054 (0.009 to 0.100)	0.020*	0.025 (-0.032 to 0.082)	0.386
Lemon/lime, yes	0.025 (-0.012 to 0.062)	0.186	0.022 (-0.017 to 0.061)	0.261	0.049 (0.001 to 0.097)	0.045*
Banana, per 100g/d	0.031 (-0.037 to 0.098)	0.378	0.035 (-0.035 to 0.106)	0.327	0.012 (-0.076 to 0.099)	0.797
Kiwi, yes	0.027 (-0.015 to 0.070)	0.204	0.008 (-0.037 to 0.052)	0.735	0.073 (0.019 to 0.128)	0.008*
Apple, per 100g/d	0.052 (0.007 to 0.096)	0.023*	0.059 (0.012 to 0.105)	0.013*	0.030 (-0.028 to 0.088)	0.311
Pear, yes	0.018 (-0.021 to 0.058)	0.357	0.011 (-0.029 to 0.052)	0.584	0.029 (-0.021 to 0.079)	0.257
Mango, yes	0.005 (-0.034 to 0.045)	0.792	0.003 (-0.038 to 0.044)	0.876	0.031 (-0.020 to 0.082)	0.229
Avocado, yes	-0.008 (-0.048 to 0.032)	0.690	-0.006 (-0.048 to 0.035)	0.765	-0.005 (-0.056 to 0.047)	0.863
Peach/nectarine, yes	0.005 (-0.033 to 0.043)	0.797	0.007 (-0.033 to 0.046)	0.740	0.014 (-0.035 to 0.063)	0.596
Apricot, yes	0.044 (-0.007 to 0.096)	0.092	0.043 (-0.011 to 0.096)	0.117	0.054 (-0.012 to 0.121)	0.108
Plum, yes	0.019 (-0.021 to 0.059)	0.344	0.015 (-0.027 to 0.056)	0.479	0.026 (-0.026 to 0.077)	0.326
Strawberry/raspberry, yes	-0.001 (-0.042 to 0.039)	0.946	-0.013 (-0.055 to 0.029)	0.538	0.043 (-0.010 to 0.095)	0.111
Grape/cherry, yes	0.038 (-0.002 to 0.078)	0.066	0.041 (-0.000 to 0.083)	0.052	0.034 (-0.018 to 0.086)	0.201
Pineapple/melon, yes	0.009 (-0.030 to 0.048)	0.654	-0.006 (-0.046 to 0.035)	0.784	0.054 (0.004 to 0.104)	0.034*
Canned fruit, yes	-0.002 (-0.049 to 0.044)	0.920	-0.015 (-0.063 to 0.033)	0.544	0.033 (-0.026 to 0.093)	0.271

a. Adjusted for energy intake, maternal age, BMI categories (>18,5, 18,5-25, 25-30, 30+), parity categories (0,1,2+), ethnicity categories (Dutch, other-western, non-western), education categories (low, intermediate, high), household income categories (<1200 per month, 1200–2000 per month > 2000 per month), marital status, smoking categories (no smoking during pregnancy, smoked until pregnancy was known, smoked during pregnancy), and season of urine collection (fall, winter, spring, summer).

b. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP, and DMP.

c. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP, and DMP.

d. Diethyl alkyl phosphates is the sum of DEDTP, DETP, and DEP.

* $p<0.05$.

Part III

Prenatal exposure to non-persistent chemicals and neurodevelopment in children

3

Chapter 3

Phthalate and bisphenol exposure during pregnancy and offspring nonverbal IQ

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Abstract

Background: Prenatal exposures to phthalates and bisphenols are associated with impaired brain development in animals. However, epidemiological studies investigating the association between prenatal phthalate or bisphenol exposure and cognition have produced mixed findings and mostly had modest sample sizes and measured the exposure during the third trimester. We examined the association between pregnancy maternal urinary biomarkers of phthalate or bisphenol exposure and nonverbal intelligence quotient (IQ) in children 6 years of age.

Method: The study sample consisted of 1,282 mother–child pairs participating in the Generation R Study, a population-based birth cohort in Rotterdam, Netherlands (enrollment 2002–2006). We measured maternal urinary concentrations of 18 phthalate metabolites and 8 bisphenols at <18, 18–25, and >25 wks of gestation. Child nonverbal IQ was measured at 6 years of age using the Snijders-Oomen Nonverbal Intelligence Test–Revised. Linear regression models were fit for each of the three collection phases separately, the three collection phases jointly, and for the averaged prenatal exposure across pregnancy.

Results: Higher urinary concentrations of phthalate metabolites during early pregnancy were associated with lower child nonverbal IQ score [e.g., B per 10-fold increase in summed low-molecular weight phthalates=−1.7 (95% CI: −3.1, −0.3)]. This association remained unchanged when adjusted for mid and late pregnancy exposures. We also observed an inverse association between late pregnancy di-n-octyl phthalate (DNOP) exposure and nonverbal IQ. Maternal urinary concentrations of bisphenols were not associated with child nonverbal IQ. There was no effect estimate modification by sex.

Conclusions: We did not observe that maternal biomarkers of bisphenol exposure are associated with nonverbal IQ. We found that phthalate exposure in early pregnancy and DNOP exposure in late pregnancy are associated with lower nonverbal IQ scores in children. Our results might suggest that particularly early pregnancy is a sensitive window of phthalate exposure, but future studies are needed to replicate our findings

Introduction

Phthalates and bisphenols are synthetic compounds incorporated in many products. For example, phthalates such as di-(2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), di-n-octyl phthalate (DNOP), and benzyl butyl phthalate (BBP) are primarily used as plasticizers for polyvinyl chloride and exist in food packaging materials, floor materials, clothing, toys, and medical devices. DBPs are also used as solvents and fixatives in paint and cosmetics.[1-3] Bisphenol A (BPA) or common replacements such as bisphenol S (BPS) and bisphenol F (BPF) exist in products such as epoxy resin coatings of canned food containers, water bottles, storage containers, thermal paper, and baby bottles.[4,5]

Phthalates and bisphenols are omnipresent in the environment, and these compounds have been detected in urine samples of mothers and their offspring.[6] Prenatal exposure to these chemicals can occur because of their ability to cross the placental and blood–brain barriers.[7-10] Studies suggest that prenatal phthalate and bisphenol exposure both interfere with the thyroid hormone system,[11-16] which is crucial for normal fetal brain development.[17] In addition, neurotoxic effects of phthalates and bisphenols may be mediated by anti-androgenic activity,[18-21] disruption of brain dopaminergic activity,[22-24] and interaction with peroxisome proliferator-activated receptors.[15,16,25]

Studies in animals have shown that prenatal phthalate and bisphenol exposure impairs neurodevelopment in the offspring.[21,24,26-34] With respect to phthalates, animal studies find inverse associations between prenatal exposure to DEHP and DBP and learning, memory, and brain development in the offspring.[24,26-29] Also, prenatal exposure to DEHP can affect developmental plasticity of the hippocampus.[30] Regarding bisphenols, animal studies have shown that gestational exposure to BPA was associated with alterations in brain morphology and brain function.[21] Further, BPA-exposed rats and mice exhibit persistent learning and memory impairments[31,33,35] and low-dose BPA exposure disrupts hippocampal CA1 neuronal morphology, which is believed to persist into adulthood.[33-35]

However, epidemiological studies investigating the associations between prenatal phthalate or bisphenol exposure and cognitive functioning in children are limited and the findings are inconclusive. One study using mother–child pairs from inner-city New York reported that higher metabolite concentrations of DBP and di-isobutyl phthalate metabolites measured in the third trimester were associated with lower intelligent quotient (IQ) score in 328 children at 7 years.[36] Similarly, other studies (n=150–417) found that higher levels of DEHP metabolites during the third trimester were associated with a lower score on the mental development index (MDI) of the Bayley Scales of Infant Development at 0.5–2 years.[37,38] Two studies found that third-trimester concentrations of DBP and mono(3-carboxypropyl) phthalate (mCPP) metabolites were inversely associated with the MDI at child age 2–3

y, but only in girls.[39,40] Another study found the averaged sum of high-molecular weight phthalates (HMWPs) and DEHP exposure across pregnancy to be inversely associated with IQ, but only in boys.[41] Yet, five other studies did not find any association between prenatal exposure to phthalates and cognition during the second[42,43] and third trimester,[43-46] using data from 100–452 mother–child pairs. Research on bisphenol exposure mostly found little evidence of an association between prenatal exposure and child cognition. One study found an inverse association between prenatal BPA exposure measured in cord blood and offspring IQ at 7 years of age in 148 children.[47] However, most other studies using maternal urine concentrations to determine exposure did not show an association between prenatal BPA exposure and cognitive functioning in 239–812 children at 1–8 years of age.[45,48-52] Further, a recent study found first trimester exposure to a mixture of 26 endocrine-disrupting chemicals to be associated with lower IQ among boys.[53] Among a broad range of chemical biomarkers, the present study included phthalate metabolites and BPA, BPF, and BPS and identified BPF as the primary chemical of concern. In addition, concentrations of BPA, mono-ethyl phthalate (mEP), and monobenzyl phthalate (mBzP) had a considerable contribution to the overall mixture effect.[53]

The heterogeneity in epidemiological results may be explained by the fact that most of these studies had modest sample sizes, which may have limited the statistical power to consistently detect adverse associations. In addition, most studies measured prenatal exposure only during the third trimester, whereas other windows of susceptibility may exist.[54] Finally, only one previous study investigated the association between prenatal exposure to BPS and BPF with IQ. To address these limitations, we studied a large cohort from the Generation R Study, which is characterized by detailed follow-up information of the child and three repeated measurements (early, mid, and late pregnancy) of urinary phthalate and bisphenol biomarkers, including BPF and BPS. We investigated the extent to which maternal exposure to phthalates or bisphenols during pregnancy are associated with offspring's nonverbal IQ at 6 years of age.

Methods

Study participants and follow-up

Generation R is a prospective population-based birth cohort designed to identify early environmental and genetic determinants of growth and development.[55] Briefly, all pregnant women who resided in the study area in Rotterdam, Netherlands, and had a delivery date between April 2002 and January 2006 were eligible. All eligible pregnant women who visited a midwife or obstetrician in Rotterdam were contacted by the Generation R Study staff for recruitment. The study staff were able to communicate with the pregnant women in Dutch, English, French, Portuguese, and Turkish. Among the 9,778 mothers

who participated in the study, 8,879 (91%) were enrolled during pregnancy and the rest were enrolled during routine visits of the newborn to the child health centers. The enrollment procedure has been previously described in detail.[56-60] Between February 2004 and January 2006, women provided spot urine specimens at the time of routine ultrasound examinations during early, mid, and late pregnancy. A total of 2,083 women provided a complete set of three urine specimens.

When the children turned 6 years of age, the families were invited to participate in an in-person follow-up visit to collect neurobehavioral data, biospecimens, and sociodemographic and health data. Of the 2,083 mother–child pairs with three urinary samples, 1,405 provided data at 6 years of age of the child. The availability of follow-up data was a requirement to allow studies of the associations between prenatal phthalate and bisphenol exposure and child health, including cognition. Of these 1,405 mother–child pairs, 1,282 had complete data on nonverbal IQ and comprised the study sample. Women in this subset had higher education and income levels, were slightly older, and were more likely to be of Dutch national origin than the broader Generation R cohort.[55]

The study protocol underwent human subjects review at Erasmus Medical Center, Rotterdam, Netherlands (MEC 198.782.2001.31, MEC-2007-413). Mothers provided written informed consent for themselves and their children.

Bisphenol and phthalate measurements in urine

Maternal spot urine specimens were collected during early (mean=13.3 wks of gestation, range=6.5–17.9 wks), mid (mean=20.4 wks of gestation, range=18.1–24.9 wks), and late pregnancy (mean=30.3 wks of gestation, range=27.4–34.5 wks). Details on urine specimen collection have been described elsewhere.[61] Briefly, all urine samples were collected (at 0800–1000 hours) in 100-mL polypropylene urine collection containers that were kept for a maximum of 20 h in a cold room (4°C) before being frozen at –20°C in 20-mL aliquots in 25-mL polypropylene vials. The urine specimens were shipped on dry ice in 4-mL polypropylene vials to the Wadsworth Center, New York State Department of Health, Albany, New York, for analysis of phthalate metabolite and bisphenol concentrations.

A detailed description of the analytical procedure is given elsewhere.[62] Briefly, quantitative detection of phthalate metabolites was achieved by using a solid-phase extraction method followed by enzymatic deconjugation of the glucuronidated phthalate monoesters coupled with high performance liquid chromatography electrospray ionization–tandem mass spectrometry (HPLC-ESI-MS/MS),[63] which allowed for the rapid detection of 18 metabolites of phthalates with limits of detection (LODs) in the range of 0.008–0.89 ng/mL. Quantitative detection of bisphenols was realized using a liquid–liquid extraction method followed by enzymatic deconjugation of the glucuronidated bisphenols coupled

with HPLC-ESI-MS/MS, which permitted the detection of eight biomarkers of bisphenols (including BPA, BPF, and BPS) with an LOD range of 0.03–0.79 ng/mL. Similarly, samples were analyzed for creatinine using HPLC-ESI-MS/MS. Quantification of calibration check standards resulted in an LOD of 0.30 ng/mL.

Exposure biomarkers were excluded from further statistical analyses if more than 80% of the study population had concentrations below the LOD. Urinary biomarkers for exposure to phthalates were grouped according to their parent compound, biologic activity, and source of exposure in order to limit multiple comparisons. Phthalate groups included low-molecular weight phthalates (LMWPs), HMWPs, DEHP, DNOP, and phthalic acid (PA). For the LMWP group, we summed mono-methyl phthalate (mMP), mEP, mono-n-butyl phthalate (mBP), and mono-isobutyl phthalate (mIBP) metabolite concentrations. For the HMWP group, we summed mono-(2-ethyl-5-carboxypentyl) phthalate (mECPP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (mEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (mEOHP), mono-[(2-carboxymethyl)hexyl] phthalate (mCMHP), mCPP, mBzP, mono-hexyl phthalate (mHxP), and mono-2-heptyl phthalate (mHpP) metabolite concentrations. For the DEHP group, we summed mECPP, mEHHP, mEOHP, and mCMHP metabolite concentrations and used MCPP metabolite concentrations as a proxy for DNOP exposure. Finally, PA is an end metabolite of all phthalates. PA metabolite concentrations were therefore analyzed separately as a proxy for total phthalate exposure. Regarding bisphenol concentrations, we calculated the sum for total bisphenol concentrations. Prior to the statistical analyses, all concentrations below the LOD were replaced with the LOD divided by the square root of 2.[64]

Nonverbal IQ at child age 6 years

Children's nonverbal IQ was assessed by administering the Mosaics and Categories subtests from the Snijders-Oomen Nonverbal Intelligence Test–Revised (SON-R), a reliable and well-validated instrument.[65-67] The correlation between the total score of the SON-R 2.5-7 and the performance IQ score of the Wechsler Preschool and Primary Scale of Intelligence has been reported to be between 0.60 and 0.83, and the average reliability of the SON-R 2½–7 IQ score was 0.90.[66-68] Further, the test is regarded as highly reliable and rated good (3 of 3) by the commission of Netherlands Institute for Psychologists. The two language-independent subtests that included items that probe visuospatial and abstract reasoning abilities were selected because of the multiethnic composition of the Generation R Study. Subtest raw test scores were converted into age-standardized nonverbal IQ scores. These standardized scores, based on the two subtests, correlated well (r=0.86) with those based on the complete instrument.[69]

Additional data collection

Maternal reproductive, sociodemographic, and cognitive data were assessed by questionnaires and/or observations. During the first prenatal visit, height and weight were measured, but prepregnancy weight was self-reported by participants. These data were used to calculate body mass index (BMI). In addition, data was collected on maternal age (years), parity (0, 1, or ≥2), smoking (no smoking during pregnancy, smoking until pregnancy recognized, and continued smoking during pregnancy), alcohol intake during pregnancy [no alcohol consumption during pregnancy, alcohol consumption until pregnancy recognized, continued occasionally (<1 glass/wk), and continued frequently (≥1 glass/wk)], marital status (married/partner or single), household total net income [<1,200 euros/month (i.e., below the Dutch social security level), 1,200–2,000 euros/month, >2,000 euros/month], highest completed education level [low (<3y at general secondary school) intermediate (≥3y of secondary education), and high (university degree or higher vocational training)], ethnicity (Dutch national origin, other-Western, and non-Western), and folic acid intake (none, started in first 10 wks of pregnancy, and started preconception). Maternal IQ was examined when mother–child pairs attended the 6-y examination and was assessed using a computerized Ravens Advanced Progressive Matrices Test, Set I.[70] The test is a 12-item reliable and validated short version of the Raven's Progressive Matrices to assess nonverbal cognitive ability.[71] The consumption of fruit and vegetables was assessed in the first trimester using a modified version of a validated food frequency questionnaire and was adjusted for energy intake.[72] Except for BMI (12%), smoking (10%), income (15%), folic acid intake (21%), and fruit and vegetable intake (25%), the percentage of missing values was below 10%.

Statistical methods

Urinary phthalate metabolite and bisphenol concentrations were expressed on a creatinine basis and log10 transformed (in micrograms per gram creatinine). Missing phthalate metabolite and bisphenol concentration values (n=13 with missing data in one or two urinary collection periods during pregnancy) and all missing covariate data were imputed 10 times with the multivariate imputation by chained equations method in R (version 3.5.3; R Development Core Team).[73,74] Urinary BPA and PA metabolite concentrations and the child nonverbal IQ score were included as predictors for the imputation of covariates. The outcome variable child nonverbal IQ was not imputed. We calculated intraclass correlation coefficients (ICCs) for individual measurements, for example, during early pregnancy (single-rater, relevant to the time-specific analyses), and for the mean of the three measurements across pregnancy (mean of k raters), using two-way mixed-effects models with absolute agreement.[75] Further, we first performed regression analyses to estimate the associations of grouped phthalate and bisphenol urinary concentrations with nonverbal IQ for each collection phase (gestational age <18, 18–25, and >25 wks). Second, a mutually adjusted model was fitted in which the association of prenatal phthalate and bisphenol

urinary concentrations from each time period on nonverbal IQ were jointly estimated. To test whether the association between prenatal urinary concentrations of phthalate metabolites and bisphenols and nonverbal IQ differed across time windows of exposure (pinteraction<0.1), we used the multiple informant method, in which different exposure windows are treated as informants.[76] We chose this strategy to identify possible windows of susceptibility and to be able to compare our results with other studies that used a single spot urine sample in pregnancy to determine phthalate and bisphenol concentrations. Third, we carried out regression analyses to estimate the association between the averaged prenatal urinary concentrations across pregnancy and nonverbal IQ. Urinary metabolite concentrations of phthalates and (especially) bisphenols vary over time. Therefore, the average is most likely a better approximation of each participant's exposure during pregnancy than any exposure measurement on its own. Fourth, we presented restrictive cubic splines for untransformed grouped biomarker concentrations that were predictive of nonverbal IQ. Finally, several studies have suggested that sex may be a potential effect estimate modifier in the association of prenatal exposure to phthalates and bisphenols and neurodevelopmental outcomes.[36,37,39-41,48-50] We therefore explored potential effect modification by sex via stratification, interaction terms, and augmented product terms (pinteraction<0.05).[77]

We present results from unadjusted and adjusted analyses. The adjustment variables were maternal age (continuous), ethnicity (categorical), education (categorical), income (categorical), marital status (categorical), alcohol consumption during pregnancy (categorical), maternal nonverbal IQ (continuous), prepregnancy BMI (continuous), parity (categorical), smoking during pregnancy (categorical), child sex (categorical), and child age at assessment (continuous). These potential confounders were selected a priori defined with a directed acyclic graph (DAG)[78] based on previous studies of prenatal phthalate and bisphenol exposure and child neurodevelopment and on biologically plausible covariate–exposure and covariate–outcome associations observed in our data (see Figure S1).

Sensitivity analyses

Several sensitivity analyses were performed. First, we used another common method to adjust for creatinine by refitting visit-specific and averaged models, with concentrations expressed in nanograms per milliliter and creatinine concentration added as a separate covariate. We performed this sensitivity analyses to facilitate comparison with previous studies investigating prenatal exposure to nonpersistent chemicals and neurodevelopment that have used this adjustment method. Second, we performed post hoc analyses in which we explored the associations of individual phthalate metabolite and bisphenol concentrations with nonverbal IQ because individual phthalates and bisphenols within the summed groups may have different neurotoxic effects. Third, we used inverse probability

weighting (stabilized weights) to correct for potential selection bias[79] and to provide results representative for the full Generation R Study cohort (n=9,901) given that children included in the analysis (n=1,282) were more likely to have parents who were of Dutch national origin, older, and from a higher socioeconomic.[55] Baseline characteristics significantly different (p<0.20) between the initially recruited cohort and the current analysis sample are presented in Table S1. Fourth, because diet and the intake of healthy nutrients may confound the association between prenatal phthalate and bisphenol exposure (e.g., food packaging) and child cognition (e.g., healthy nutrients), we performed a sensitivity analyses in which we additionally adjusted for maternal fruit, vegetables, and folic acid intake. Fifth, to correct for multiple hypothesis testing, each p-value was compared with a threshold, defined as 0.05 divided by the effective independent number of tests.[80] The corrected P-value was calculated based on the correlation structure between the phthalate metabolite groups (LMWP, HMWP, DEHP, DNOP, and PA) for each time point separately (corrected p<18 wks of gestation=0.02, 18–25 wks of gestation=0.018, >25 wks of gestation=0.017), and for the average exposures (corrected p=0.017).

Results

Sample characteristics

At enrollment, most of the participating women were between 30 and 35 years of age (43%), nulliparous (61%), Dutch (54%), married (89%), and highly educated (50%) (Table 1). A large group had a prepregnancy BMI of between 18.5 and 25 (70%), a high income (68%), and did not consume alcoholic beverages (42%) or smoke (75%) during pregnancy.

Phthalate and bisphenol concentrations

The median LMWP metabolite concentrations for <18, 18–25, and >25 wks of gestation were 240, 103, and 232μg/g creatinine, respectively (Table 2). The median HMWP measured at <18, 18–25, and >25 wks of gestation were 69, 33, and 52μg/g creatinine. Total bisphenol concentrations comprised mostly BPA, and the median total bisphenol concentrations for <18, 18–25, and >25 wks of gestation were 2, 1, and 2μg/g creatinine, respectively. Descriptive statistics of the individual biomarkers from our study sample can be found in Tables S2 and S3. The ICC for the grouped phthalate metabolite concentrations varied between 0.2 and 0.4 for a single measurement and varied between 0.4 and 0.6 for the mean of the three measurements (see Table S4). Regarding the bisphenol group, the ICCs for a single-measurement (0.05) and for the mean of the three measurements (0.14) were poor.

Table 1. Characteristics of study participants (n=1282).

		Phthalic acid [a]		Bisphenols [b]		Non-verbal IQ [c]
Maternal characteristics [d]	Percentages	Median	P25, P75	Median	P25, P75	Mean ± SD
Age in years						
< 20	2.0	109.2	65.4, 162.5	1.5	1.2, 2.6	94 ± 15
20-< 25	11.5	85.5	61.9, 123.9	1.7	1.1, 2.8	94 ± 14
25-< 30	26.8	82.6	52.1, 129.7	1.7	1.1, 2.8	102 ± 15
30-< 35	42.9	84.7	52.5, 131.8	1.9	1.3, 2.9	104 ± 16
≥ 35	16.8	85.8	54.5, 135.5	1.9	1.1, 2.9	104 ±13
Pre-pregnancy body mass index (BMI) (kg/m^2)						
< 18.5	2.5	88.6	45.4, 123.5	1.9	1.3, 3.1	102 ± 16
18.5-< 25	69.8	79.9	51.1, 124.3	1.8	1.1, 2.8	103 ± 15
25-< 30	19.1	95.6	58.2, 146.0	2.1	1.3, 3.3	100 ± 15
≥ 30	8.6	95	67.7, 142.0	1.8	1.2, 3.0	98 ± 17
Parity						
0	60.5	86	54.0, 133.9	1.8	1.2, 2.8	103 ± 15
1	28.2	79.9	52.5, 119.3	1.9	1.2, 3.0	102 ± 15
≥ 2	11.3	88.2	54.2, 139.8	1.7	1.1, 2.8	98 ± 15
Ethnicity						
Dutch	53.9	81.1	50.4, 131.8	1.9	1.2, 2.9	106 ± 15
Other Western	12.4	78.2	52.9, 128.6	1.7	1.2, 2.5	102 ± 14
Non-Western	33.7	88.3	58.7, 132.9	1.8	1.1, 2.9	96 ± 15
Education [e]						
Low	18.9	95.2	65.0, 151.7	1.8	1.2, 2.8	96 ± 15
Intermediate	30.7	85.9	55.3, 137.0	1.9	1.2, 3.1	100 ± 14
High	50.4	76.2	48.8, 122.1	1.8	1.1, 2.8	106 ± 14
Household income in Euros						
<1200 per month	15.4	91.1	62.4, 135.9	1.6	1.1, 2.8	95 ± 15
1200–2000 per month	17.0	86.9	56.2, 137.3	1.9	1.3, 3.0	100 ± 15
> 2000 per month	67.6	81.2	50.7, 127.4	1.8	1.2, 2.9	105 ± 14
Marital status						
Married/ living with partner	88.5	82.8	52.0, 128.9	1.8	1.2, 2.9	103 ± 15
No partner	11.5	96.2	67.7, 151.0	1.8	1.1, 3.1	99 ± 15
IQ-score						
≤ 85	22.5	91.3	58.3, 145.5	1.9	1.1, 2.9	96 ± 15
>85-≤ 100	43.3	86.9	55.6, 134.8	1.8	1.2, 2.7	102 ± 14
>100 -< 115	17.9	79.3	50.8, 125.2	1.8	1.2, 2.8	105 ± 14
≥ 115	16.3	72.9	47.3, 118.6	1.9	1.3, 3.1	107 ± 16
Smoking						
No smoking during pregnancy	75.3	78.7	51.5, 122.7	1.8	1.1, 2.8	103 ± 15
Until pregnancy recognized	10.2	97.6	62.8, 140.1	2.0	1.4, 3.2	101 ± 14
Continued during pregnancy	14.5	105.6	71.2, 154.8	1.9	1.2, 3.1	97 ± 15
Alcoholic beverage consumption						
No alcohol consumption	42.1	86.5	56.6, 137.6	1.8	1.1, 2.8	99 ± 15
Until pregnancy recognized	17.0	78.5	52.4, 132.7	1.7	1.1, 2.7	103 ± 14
Continued occasionally [f]	35.2	85.5	51.3, 124.9	2.0	1.3, 3.0	105 ± 16
Continued frequently [g]	5.8	74.6	48.1, 129.7	2.0	1.4, 3.1	105 ± 14
Infant characteristics [d]	Percentages	Median	P25, P75	Median	P25, P75	Mean ± SD
Sex of infant at birth						
Male	50.5	84.5	52.6, 130.7	1.9	1.2, 3.0	102 ± 16
Female	49.5	84.1	53.7, 132.3	1.7	1.2, 2.7	101 ± 15

Continue

Continued

Note: BMI, body mass index; IQ, intelligence quotient; P, percentile; SD, standard deviation.
a. Average total phthalic acid in ug/g creatinine by category of characteristics.
b. Average total bisphenols in ug/g creatinine by category of characteristics.
c. Non-verbal IQ by category of characteristics.
d. There were missing observations for BMI (n=157), parity (n=8), ethnicity (n=13), education (n=60), household income (n=193), marital status (n=69), maternal IQ (n=34), smoking (n=132), alcohol consumption (n=91).
e. Low: No education finished, Primary education, lower vocational training, intermediate general school or <3 years at general secondary school. Intermediate: +3 years of secondary education, Intermediate vocational training or first year of higher vocational training. High: University degree or higher vocational training.
f. Less than 1 glass/week.
g. One or more glass/week for at least two trimesters.

Associations with nonverbal IQ

Creatinine-adjusted LMWP, DNOP, and PA metabolite concentrations at <18 wks of gestation were significantly associated with child nonverbal IQ score (Table 3). For example, a 10-fold higher LMWP, DNOP, or PA metabolite concentration was associated with lower nonverbal IQ scores of 1.7 points [95% confidence interval (CI): −3.1, −0.3], 2.0 points (95% CI: −3.7, −0.2), and 1.9 points (95% CI: −3.6, −0.3), respectively. The associations for HMWP [B=−1.8 (95% CI: −3.6, 0.0)], and DEHP [B=−1.7 (95% CI: −3.5, 0.1)] metabolite concentrations with nonverbal IQ were comparable in magnitude. When adjusted for mid and late pregnancy exposures, the associations of child nonverbal IQ with grouped phthalate metabolite concentrations were similar. Mid and late pregnancy concentrations of grouped phthalate metabolites were generally not significantly associated with nonverbal IQ. However, a 10-fold increase in DNOP metabolite concentration at >25 wks of gestation was associated with a 2.4-point lower nonverbal IQ score (95% CI: −4.8, 0.0). Further, the significant interaction terms between LMWP, HMWP, DNOP, and PA metabolite concentrations and timing of exposure (p=0.06, p=0.09, p=0.03, and p=0.05, respectively) suggested that the potential effects of prenatal LMWP, HMWP, DNOP, and PA metabolite concentrations on nonverbal IQ might differ depending on the timing of exposure. No significant interaction terms were observed for DEHP metabolite concentrations and timing of exposure. Further, compared with the associations observed at <18 wks of gestation, we observed similar estimates in terms of magnitude for averaged LMWP [B=−1.7 (95% CI: −3.7, 0.4)] and PA [B=−1.8 (95% CI: −4.6, 1.0)] metabolite concentrations, and a greater estimate in terms of magnitude for averaged DNOP metabolite concentrations [B=−3.0 (95% CI: −6.0, 0.0)]. Finally, representative restrictive cubic splines for untransformed grouped phthalate metabolite concentrations at <18 wks of gestation and DNOP metabolite concentrations at >25 wks of gestation (see Figure S2) indicated a slightly steeper inverse association between exposure and outcome at lower levels of exposure.

Table 2. Descriptive statistics of creatinine adjusted phthalate and bisphenol concentrations [a] in urine samples measured in pregnancy.

	N	min	P25	P50	P75	max
Phthalate metabolite concentrations in ug/g creatinine [b]						
LMWP <18 weeks	1274	0.4	109.5	239.5	603.3	102789.2
LMWP 18-25 weeks	1270	4.3	46.8	102.6	238.0	36050.2
LMWP >25 weeks	1269	21.1	103.0	232.3	530.5	8131.3
HMWP <18 weeks	1274	0.6	39.6	68.5	126.6	2837.4
HMWP 18-25 weeks [c]	1270	2.3	19.3	33.1	59.0	15577.1
HMWP >25 weeks [c]	1269	3.6	35.0	52.3	81.9	1683.6
DEHP <18 weeks	1274	0.3	31.4	55.2	103	2821.2
DEHP 18-25 weeks	1270	1.8	14.4	25.0	46.3	15574.4
DEHP >25 weeks	1269	3.2	29.6	45.0	71.0	1673.5
DNOP <18 weeks	1274	0	0.9	1.6	2.8	106.5
DNOP 18-25 weeks	1270	0	0.4	0.8	1.4	65.8
DNOP >25 weeks	1269	0	1.2	1.9	3.0	72.0
PA <18 weeks	1274	0.6	33.8	63.8	123.4	12433.4
PA 18-25 weeks	1270	4.3	56.5	111.6	250.1	2885.2
PA >25 weeks	1269	2.6	44.2	73.9	127.1	1562.4
Total bisphenol concentrations in ug/g creatinine						
<18 weeks [d]	1274	0.1	1	2.1	5.2	982.8
18-25 weeks [e]	1270	0	0.6	1.2	2.5	277.6
>25 weeks [f]	1269	0.1	1.1	2.0	4.2	145.4

Abbreviations: n=number, min=minimum, P=percentile, max=maximum

a. Concentrations below the limit of detection(LOD) were imputed with LOD/√2.

b. Phthalate metabolites are grouped into: low molecular weight phthalate metabolites (LMWP) = sum of Monomethylphthalate, Monoethylphthalate, Mono-isobutylphthalate, and Mono-n-butylphthalate, high molecular weight phthalate metabolites (HMWP) = sum of mono-(2-ethyl-5-carboxypentyl) phthalate, mono-(2-ethyl-5-hydroxyhexyl)phthalate, mono-(2-ethyl-5-ox-ohexyl) phthalate, mono-[(2-carboxymethyl)hexyl]phthalate, mono(3-carboxypropyl) phthalate, monobenzylphthalate, mono-hexylphthalate, and mono-2-hep-tylphthalate, Di-2-ethylhexylphthalate metabolites (DEHP) = sum of Mono-(2-ethyl-5-carboxypentyl)phthalate, Mono-(2-ethyl-5-hydroxyhexyl)phthalate, Mono-(2-ethyl-5-oxohexyl)phthalate, and Mono-[(2-carboxymethyl)hexyl]phthalate, Di-n-octylphthalate metabolites (DNOP) = Mono(3-carboxypropyl)phthalate, Phthalic acid (PA) = a proxy for total phthalate exposure measured in ug/g creatinine.

c. with the exclusion of mono-hexylphthalate, and mono-2-hep-tylphthalate metabolite concentrations

d. Total bisphenol =-sum of Bisphenol A, bisphenol F, and Bisphenol S.

e. Total bisphenol =-sum of Bisphenol A, and Bisphenol S.

f. Total bisphenol =-sum of Bisphenol A and bisphenol F.

Table 3. Difference in child non-verbal IQ score at age six years per log10 increase in creatinine adjusted maternal urine phthalate metabolite and bisphenol concentrations, by timing of urine sampling.

		Unadjusted		Adjusted [a]		Mutually adjusted [b]	
Phthalate metabolites [c]		B	95%CI	B	95%CI	B	95%CI
Σ LMWP metabolites							
	<18 weeks	-3.21	-4.64, -1.78	-1.68	-3.05, -0.32	-1.75	-3.21, -0.29
	18-25 weeks	-1.70	-3.30, -0.11	0.27	-1.25, 1.79	1.04	-0.58, 2.67
	>25 weeks	-2.85	-4.52, -1.18	-1.07	-2.65, 0.51	-0.80	-2.50, 0.90
	Averaged	-4.85	-6.96, -2.75	-1.68	-3.72, 0.36	-	
	p [d]						0.06
Σ HMWP metabolites							
	<18 weeks	-3.63	-5.55, -1.70	-1.80	-3.62, 0.03	-1.98	-3.82, -0.13
	18-25 weeks [e]	-0.87	-2.81, 1.07	1.04	-0.80, 2.87	1.19	-0.67, 3.05
	>25 weeks [e]	-0.14	-2.82, 2.54	0.73	-1.79, 3.26	0.79	-1.76, 3.33
	Averaged	-4.41	-7.69, -1.12	-0.37	-3.52, 2.78	-	
	p [d]						0.09
Σ DEHP metabolites							
	<18 weeks	-3.20	-5.09, -1.31	-1.73	-3.52, 0.05	-1.89	-3.69, -0.09
	18-25 weeks	-0.42	-2.33, 1.49	0.92	-0.88, 2.72	1.06	-0.76, 2.88
	>25 weeks	0.20	-2.38, 2.77	0.65	-1.78, 3.09	0.71	-1.75, 3.16
	Averaged	-2.21	-4.64, 0.22	0.05	-2.26, 2.36	-	-
	p [d]						0.11
Σ DNOP metabolites							
	<18 weeks	-2.61	-4.46, -0.76	-1.98	-3.72, -0.23	-1.86	-3.65, -0.08
	18-25 weeks	-0.13	-2.20, 1.94	0.38	-1.57, 2.32	0.87	-1.1, 2.84
	>25 weeks	-1.10	-3.64, 1.43	-2.39	-4.76, -0.02	-2.10	-4.51, 0.30
	Averaged	-3.28	-6.47, -0.09	-2.97	-6.00, 0.06	-	
	p [d]						0.03
PA metabolites							
	<18 weeks	-2.59	-4.32, -0.87	-1.93	-3.55, -0.31	-1.90	-3.55, -0.25
	18-25 weeks	-0.90	-2.72, 0.91	0.85	-0.87, 2.57	1.07	-0.66, 2.80
	>25 weeks	-2.46	-4.75, -0.17	-1.09	-3.25, 1.07	-0.78	-2.98, 1.42
	Averaged	-4.64	-7.57, -1.71	-1.77	-4.57, 1.04	-	
	p [d]						0.05
		Unadjusted		Adjusted [a]		Mutually adjusted [b]	
Bisphenol metabolites		B	95%CI	B	95%CI	B	95%CI
	<18 weeks [f]	0.38	-1.10, 1.86	0.49	-0.90, 1.87	0.48	-0.90, 1.87
	18-25 weeks [g]	0.48	-1.19, 2.15	0.13	-1.44, 1.70	0.12	-1.44, 1.69
	>25 weeks [h]	1.05	-0.74, 2.85	0.05	-1.62, 1.72	0.06	-1.61, 1.74
	Averaged	1.81	-1.03, 4.65	0.76	-1.89, 3.42	-	
	p [d]						0.92

a. Adjusted for maternal age, maternal non-verbal IQ, sex of child, age of the child at assessment, ethnicity, education, income, marital status, maternal alcohol consumption, BMI, parity categories, and smoking.
b. Adjusted model with the inclusion of the three exposures in one model.
c Phthalate metabolites are grouped into: low molecular weight phthalate metabolites (LMWP) = sum of Monomethylphthalate, Monoethylphthalate, Mono-isobutylphthalate, and Mono-n-butylphthalate, high molecular weight phthalate metabolites (HMWP) = sum of mono-(2-ethyl-5-carboxypentyl) phthalate, mono-(2-ethyl-5-hydroxyhexyl)phthalate, mono-(2-ethyl-5-ox-ohexyl) phthalate, mono-[(2-carboxymethyl)hexyl]phthalate, mono(3-carboxypropyl) phthalate, monobenzylphthalate, mono-hexylphthalate, and mono-2-hep-tylphthalate, Di-2-ethylhexylphthalate metabolites (DEHP) = sum of Mono-(2-ethyl-5-carboxypentyl)phthalate, Mono-(2-ethyl-5-hydroxyhexyl)phthalate, Mono-(2-ethyl-5-oxohexyl)phthalate, and Mono-[(2-carboxymethyl)hexyl]phthalate, Di-n-octylphthalate metabolites (DNOP) = Mono(3-carboxypropyl)phthalate, Phthalic acid (PA) = a proxy for total phthalate exposure measured in ug/g creatinine.
d. Tests whether exposure from different time points relates in the same manner to non-verbal IQ scores using the multiple informant method (Sanchez et al. 2011).
e. with the exclusion of mono-hexylphthalate, and mono-2-hep-tylphthalate metabolite concentrations
f. Total bisphenol =-sum of Bisphenol A, bisphenol F, and Bisphenol S.
g Total bisphenol =-sum of Bisphenol A, and Bisphenol S.
h. Total bisphenol =-sum of Bisphenol A and bisphenol F.

Effect estimate modification by sex

No effect estimate modification by sex was observed for the association between maternal urinary concentrations of grouped phthalate metabolite concentrations and nonverbal IQ (see Table S5). Similarly, there was no effect estimate modification by sex for the association between maternal urinary bisphenol concentrations and nonverbal IQ.

Sensitivity analyses

First, the results with concentrations expressed as nanograms per milliliter and creatinine concentration added as a separate covariate were similar to the results of the main analyses using creatinine-corrected concentrations (see Table S6). Second, associations between individual urine phthalate metabolite concentrations at <18 wks of gestation and nonverbal IQ scores were inverse for all metabolites except for mHxP. Next, associations with mEP, mCMHP, and mCPP remained significant after confounder adjustment (Figure 1; see also Table S7). For example, the mean nonverbal IQ score was 1.3 points lower (95% CI: –2.4, –0.2) in association with a 10-fold increase in urine mEP concentration at <18 wks of gestation. Most mid and late pregnancy concentrations of phthalate metabolites were

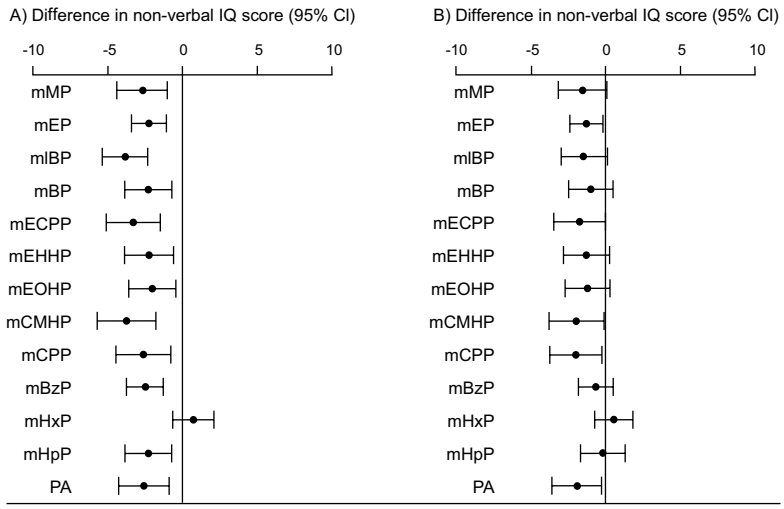

Figure 1. Difference in non-verbal IQ with a log10 increase in phthalate metabolite concentrations in ug/g creatinine at <18 weeks of gestation. Corresponding numeric data are reported in Table S7. **A)** Unadjusted model. **B)** Adjusted for maternal age, maternal non-verbal IQ, sex of child, age of the child at assessment, ethnicity, education, income, marital status, maternal alcohol consumption, BMI, parity categories, and smoking categories. mMP= mono-methyl phthalate, mEP= mono-ethyl phthalate, mIBP= mono-isobutyl phthalate, mBP= mono-n-butyl phthalate, mECPP= mono- (2-ethyl-5-carboxypentyl) phthalate, mEHHP= mono- (2-ethyl-5-hydroxyhexyl) phthalate, mEOHP= mono-(2-ethyl-5-oxohexyl) phthalate, mCMHP= mono-[(2-carboxymethyl)hexyl]phthalate, mCPP= mono(3-carboxypropyl) phthalate, mBzP= monobenzyl phthalate, mHxP= mono-hexylphthalate, mHpP= mono-2-heptylphthalate, PA= phthalic acid. Abbreviation

not significantly associated with nonverbal IQ. However, a 10-fold increase in mCPP metabolite concentrations at >25 wks of gestation was associated with a 2.4-point lower nonverbal IQ score (95% CI: −4.8, 0.0). As compared with the associations observed at <18 wks of gestation, when we observed similar estimates in terms of magnitude for averaged mEP and mCMHP metabolite concentrations as well as a greater estimate in terms of magnitude for averaged mCPP metabolite concentrations. No associations were observed for the BPA, BPS, and BPF. Third, the results using inverse probability weighting to correct for potential selection bias (see Table S8) and the results in which we additionally adjusted for diet (see Table S9) were similar to the main results. Fourth, when the main results were corrected for the effective independent number of tests, the adjusted and mutually adjusted associations of maternal LMWP metabolite concentrations at <18 wks of gestation with nonverbal IQ remained significant. Similarly, the adjusted association between PA metabolite concentrations at <18 wks of gestation with nonverbal IQ survived the multiple testing correction.

Discussion

In this large population-based study, we consistently observed that early pregnancy phthalate exposure was associated with lower child nonverbal IQ scores at 6 years of age. Urinary metabolite concentrations of early pregnancy LMWP, HMWP, DEHP, DNOP, and PA were associated with lower nonverbal IQ. We also found an inverse association between late pregnancy DNOP exposure and nonverbal IQ. Our findings did not support an association between other mid and late pregnancy grouped phthalate exposures, and we found little or no evidence of an association between prenatal bisphenol exposure and child nonverbal IQ. Finally, our results did not support effect modification by sex.

The results from our study may help clarify the so-far inconclusive results of epidemiological studies investigating the effect of prenatal exposure to phthalates on cognitive functioning. To our knowledge, most other previous studies have examined phthalate exposure during mid or late pregnancy. We did not find clear evidence for an association between mid and late pregnancy phthalate exposure and offspring IQ. Consistent with our results, previous studies have also found that prenatal phthalate metabolite concentrations measured at 14–27 wks of gestation,[42] at 22–29 wks of gestation,[81] and at 26–36 wks of gestation[82] were not associated with IQ score measured in children. Similarly, Li et al. (2019) assessed the association between mid (range: 10–23 wks of gestation) and late pregnancy (19–35 wks of gestation) biomarkers of phthalate exposure and child IQ and did not find an association for most phthalate metabolite concentrations. However, they did report an inverse association for mid mBzP metabolite concentrations. Moreover, contrary to our results, several studies found third-trimester LMWP, DEHP, and HMWP exposure to

be associated with cognition. A study demonstrated that a log-unit higher mBP and mIBP metabolite concentrations resulted in 2.7 lower IQ scores [mBP= (–2.7, 95% CI: –4.3, –1.1), mIBP= (–2.7, 95% CI: –4.2, –1.2)] in children 7 years of age.[36] Other studies using the Bayles scales have estimated that higher DEHP and HMWP exposure was predictive of cognitive functioning in children 6 months to 3 years of age.[37,38] Next, in contrast to previous studies examining DNOP exposure in late pregnancy,[40,43,83] we found an inverse association with late pregnancy mCPP metabolite concentrations and cognition. Differences in results may be due to ethnic and socioeconomic differences and differences in cognitive measurement scales. For example, the study population of Factor-Litvak et al. (2014) consisted of Hispanic and African American women of inner-city New York with low socioeconomic status. Our study population comprised mainly Dutch participants and fewer socioeconomically deprived persons, limiting comparability of the results. Further, the Bayley Scales of Infant Development measures cognition through evaluation of sensory perception, knowledge, memory, problem solving, and early language at a young age. We used the SON-R test to measure cognition at 6 years of age, which is an age when IQ is arguably more stable than in infants or toddlers.

Regarding bisphenols, our results are in line with those of previous studies that also were unable to detect an association between maternal urinary BPA concentrations and cognitive functioning in children.[49-52,81] BPA concentrations measured at 12 wks of gestation,[52] at 16–26 wks of gestation,[50,51] and at 22–29 wks of gestation[45] were not associated with IQ scores measured in children 1 and 8 years of age. Casas et al. (2015) used the Bayley Scales (at 1 year of age) and the McCarthy Scales of Children's Abilities (at 4 years of age) to assess cognitive functioning but found no association for averaged first- and third-trimester BPA exposure. However, one study assessing BPA exposure using cord blood instead of maternal urine estimated an inverse association with IQ in children at 7 years of age.[47]

To our knowledge, only one previous study investigated prenatal BPS and BPF exposure in relation to cognitive functioning in children.[53] The present study examined early pregnancy exposure to a mixture of endocrine-disrupting chemicals, including BPA, BPF, and BPS, and identified BPF as the primary chemical with a substantial contribution to the overall mixture effect on lower IQ among boys.[53] Manufacturers seeking BPA alternatives have turned to other bisphenols to produce BPA-free products.[84] However, experimental studies have shown that the BPA replacements have metabolism, potencies, and mechanisms of action that may be similar to that of BPA.[85] BPF and BPS also display endocrine-disruptive properties.[86,87] Further, an experimental study suggested that prenatal BPS exposure is able to induce hypothalamic neurogenesis.[88] However, we did not find that prenatal exposure to BPF and BPS was associated with nonverbal IQ in children.

Several studies have estimated sex differences in the association between prenatal phthalates or bisphenol exposure with neurobehavioral problems such as aggression, hyperactivity, inattention, emotional reactiveness, orientation, motor performance, anxiety, and depression and reduced masculine play in boys.[48,89-96] In some studies of cognitive outcomes, effect modification by sex was estimated in the association of prenatal phthalate exposure and the Bayley Scales[39,40,83,97] and IQ,[41] but the findings are inconsistent regarding the exact phthalate metabolites, timing of exposure, and the sex-specific effect of the associations.

The phthalate and bisphenol concentrations in the present study were generally of the same magnitude as those reported by several other studies of pregnant women. For example median mEHHP (6–12 ng/mL), mEOHP (7–8 ng/mL), mBP (10–16 ng/mL), and BPA (1.1–1.5 ng/mL) concentrations in the present study were similar to those estimated in Canada (9, 6, 12, and 1 ng/mL, respectively)[98] and Israel (6, 5, 10, and 2 ng/mL, respectively).[99] Concentrations measured in pregnant women from Korea, China, Taiwan, and the United States were, in general, somewhat higher.[36-39,41,42,100] The difference in exposure levels between studies may be due to different dietary habits, methods and timing of urine sampling, product usage, and metabolic rate.

We found substantial confounding (~50%) of point estimates when adjusting for confounders and little change in the estimates when accounting for the exposure of other time windows. This suggests that the total effect of early pregnancy phthalate exposure on nonverbal IQ is mostly driven by the direct effect rather than by mid and late pregnancy phthalate exposure and that the effect of late pregnancy DNOP exposure is somewhat confounded by early and mid-pregnancy exposure.

The critical windows of fetal neurodevelopment toxicity for phthalate exposure is uncertain. To the best of our knowledge, this is one of the first studies among humans that investigated early pregnancy phthalate exposure in relation to offspring IQ. Our results suggest that early pregnancy might be a critical period for potential effects of phthalate exposure on cognitive development. Although biological mechanisms that might contribute to associations are uncertain, animal studies have shown that phthalates may interfere with processes essential for the development of the fetal brain.[15,16] A potential mechanism is via the disruption of the thyroid function.[15,16] Thyroid hormones are important for fetal neurodevelopment from early pregnancy onward. Animal studies have showed that thyroid hormones are involved in neocorticogenesis and the development of the hippocampus and cytoarchitecture of the somatosensory cortex.[101,102] In addition, data from animal studies have shown that maternal hypothyroxinemia interferes with neuronal migration, differentiation, synaptogenesis, and cortical layer formation.[103,104] During early gestation, the fetus depends fully on maternal thyroid hormones that cross the placenta because the fetal thyroid function does not start before 12–14 wks of pregnancy.[103,105] Further,

after the onset of fetal thyroid hormone production, the fetus remains dependent on maternal thyroid hormones.[103] Prenatal exposure to phthalates has been associated with changes in circulating thyroid hormone and low thyroid function in pregnant women, including during early pregnancy,[13,106-108] which is an important determinant of offspring neurodevelopment.[109,110] For example, earlier studies in the same cohort as the present study (the Generation R Study cohort) found maternal thyroid function during early pregnancy to be associated with nonverbal IQ.[111]

Another potential mechanism might be that prenatal phthalate exposure may affect neurodevelopment through interaction with peroxisome proliferator-activated receptors, a class of nuclear receptors involved in many physiologic processes central to neurodevelopment, including cellular reproduction and differentiation.[15,16] Animal studies have shown that phthalates may induce peroxisome proliferator-activated receptor overexpression, resulting in apoptosis of undifferentiated neurons.[112] Further, prenatal phthalate exposures may be associated with fetal growth[113] which is a strong predictor of neurodevelopment.[114] It is conceivable that fetal exposure to phthalates may affect neurodevelopment via growth restriction. However, recent reviews do not provide a clear conclusion about the effects of phthalates on pregnancy outcomes such as gestational age, birth weight, and preterm birth.[113,115,116] Several phthalates are anti-androgenic, resulting in circulating testosterone and male reproductive tract abnormalities.[117-122] Gonadal hormones are important for sex-specific brain development, and they also play a crucial role in adolescent brain remodelling.[123,124] Other potential mechanisms include the disruption of calcium signaling and lipid metabolism, which are essential for normal neurodevelopmental processes in fetal life.[15,16]

In the present study, we estimated that a 10-fold increase in early pregnancy phthalate metabolite concentrations and late pregnancy DNOP exposure was associated with 1.7–2.4 lower IQ points in children. Higher child IQ is associated with healthier behavior and lifetime achievements (including educational achievement, well-paid employment, enhanced social status, and the accompanying benefits to health) later in life.[125,126] Several studies have estimated the socioeconomic impact of IQ loss. The burden and disease costs of exposure to chemicals has been estimated to be high.[127] For example, every IQ point lost from the U.S. average is estimated to have an annual cost of US$71 billion.[128]

A strength of the present study is the large sample size. The sample of our study was approximately two to three times larger than the abovementioned studies that investigated prenatal phthalates or bisphenol exposure and cognitive function in children. Another strength of the present study is the availability of more phthalate and bisphenol biomarkers as compared with previous studies investigating prenatal exposure to phthalates or bisphenols and neurodevelopment. Finally, the three repeated measures for exposure

estimation in three time windows across pregnancy is yet another strength. This allowed us to investigate potential windows of susceptibility.

Our study has a few limitations that need to be considered. Although we adjusted for many potential confounders, we cannot rule out residual confounding by unknown unobserved background risk factors related to the likelihood of exposure and cognitive functioning. Second, we used three spot urine samples during early, mid, and late pregnancy for measurements of chemicals. Although this is more frequent than most other studies investigating prenatal exposure to phthalates and bisphenols and cognitive functioning, misclassification of exposure due to the limited number of samples may have occurred and could have resulted in less precise exposure–response estimates.[129,130] This is particularly relevant for the time-specific analyses in which we relied on a single biomarker to estimate exposure. Phthalates and bisphenols have a short half-life and are quickly metabolized in the human body. Therefore, the use of multiple pooled urine specimens across trimesters is suggested to avoid exposure misclassification.[129-131] Another limitation of the present study is the absence of information about the exact time of spot urine sampling. Because the urine spot samples were collected between 0800 and 1000 hours, there may have been a combination of first morning and random spot samples. Concentrations of chemicals, urine volume, and the rate of excretion vary with fluid intake, time of day, and other factors.[132-134] Although time of sample collection is unlikely to confound the association between phthalate and bisphenol exposure and nonverbal IQ, the difference in concentrations between morning and random spot urine could have increased the intra-individual variability. We used creatinine adjustment to account for urine dilution, which is advantageous because of its ease of measurement and the low cost and widespread availability of assays.[135] However, creatinine excretion rates may vary across pregnancy[136-138] and studies have suggested that specific gravity rather than creatinine adjustment may be more appropriate in populations undergoing physiological changes in renal function, such as pregnant women.[139,140] For example, specific gravity has a slightly better within-person reproducibility and the least amount of systematic variation when compared with creatinine adjustment [140]. However, high correlations (>0.8) between creatinine and specific gravity in spot urines have been reported.[141-143] Finally, the Generation R Study is representative of an urban population with varying ethnicities, socioeconomic statuses, and educational levels, and therefore less generalizable to populations where the phthalate and bisphenol exposure sources may differ.

In the present study, we did not observe that maternal biomarkers of bisphenols are associated with lower nonverbal IQ. We did observe that phthalate exposure in early pregnancy and DNOP exposure in late pregnancy is associated with lower nonverbal IQ scores in children. Our results might suggest that particularly early pregnancy is a sensitive window of phthalate exposure, but future studies are needed to replicate our findings.

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Supplemental material

Table S1. Variables used in logistic regression model to calculate inverse probability of attrition weights.

Variables	Explored [a]	Included [b]
Maternal educational level	x	x
Maternal ethnicity	x	x
Maternal age	x	x
Maternal parity	x	x
Maternal alcohol use during pregnancy	x	x
Maternal tobacco use during pregnancy	x	x
Maternal body mass index	x	x
Household income during pregnancy	x	x
Marital status during pregnancy	x	
Child's sex	x	
Child's birth weight	x	
Gestational age at birth	x	x

a. Characteristics explored in logistic regression models to test whether they differ between included and not-included participants of the Generation R Study cohort.
b. Characteristics that were predictive (P<0.20) of being included in our study.

Table S2. Descriptive statistics of urinary phthalate metabolite concentrations in ng/ml [a] across pregnancy measured in 1,282 women

	N	LOD	<LOD	min	P25	P50	P75	max
LMWP concentrations								
mMP <18 weeks	1274	0.06	0.2%	0	2.7	5.4	9.8	1231.8
mMP 18-25 weeks	1270	0.06	0.2%	0	1.9	3.5	6.4	2486.5
mMP >25 weeks	1269	0.06	0.5%	0	2.0	4.1	8.1	1660
mEP <18 weeks	1274	0.06	0.1%	0	40.5	139.2	484.5	27649.1
mEP 18-25 weeks	1270	0.06	0.0%	1.5	25.1	72.6	227.3	27602.6
mEP >25 weeks	1269	0.06	0.0%	1.4	44.7	127.8	410.9	17428.4
mBP <18 weeks	1274	0.14	0.7%	0.1	7.0	16.1	31.1	2715.6
mBP 18-25 weeks	1270	0.14	0.0%	0.2	5.5	9.7	19.1	23148.1
mBP >25 weeks	1269	0.14	0.2%	0.1	6.1	12.1	25.1	2573.4
mIBP <18 weeks	1274	0.09	0.2%	0.1	9.6	21.4	47.0	1826.6
mIBP 18-25 weeks	1270	0.09	0.0%	0.5	4.6	9.0	18.4	1773.7
mIBP >25 weeks	1269	0.09	0.2%	0.1	9.4	18.1	38.3	2057.1
HMWP concentrations								
DEHP								
mECPP <18 weeks	1274	0.29	0.2%	0.2	8.3	16.3	31.3	942.0
mECPP 18-25 weeks	1270	0.29	0.1%	0.2	5.9	10.7	20.7	93301.2
mECPP >25 weeks	1269	0.29	0.0%	0.7	9.4	18.2	34.3	578.5
mEHHP <18 weeks	1274	0.08	0.2%	0.1	5.8	11.8	22.7	548.8
mEHHP 18-25 weeks	1270	0.08	0.1%	0.1	3.0	5.6	11.0	273.1
mEHHP >25 weeks	1269	0.08	0.2%	0.1	5.2	10.3	19.7	336.9
mEOHP <18 weeks	1274	0.04	0.0%	0.1	3.5	7.7	15.1	416.5
mEOHP 18-25 weeks	1270	0.04	0.0%	0.2	3.7	7.5	16.6	11831
mEOHP >25 weeks	1269	0.04	0.0%	0.4	3.9	7.2	14.0	260.1
mCMHP <18 weeks	1274	0.04	0.1%	0	7.6	14.0	26.5	2729.6
mCMHP 18-25 weeks	1270	0.04	0.2%	0	2.3	4.2	7.5	5497.2
mCMHP >25 weeks	1269	0.04	1.1%	0	1.9	3.5	6.5	165.0
DINP								
mINP <18 weeks	1274	0.18	85.5%	0.1	0.1	0.1	0.1	16.1
mINP 18-25 weeks	1270	0.18	98.6%	0.1	0.1	0.1	0.1	0.6
mINP >25 weeks	1269	0.18	99.9%	0.1	0.1	0.1	0.1	16.9
DIDP								
mIDP <18 weeks	1274	0.89	92.6%	0.6	0.6	0.6	0.6	14.2
mIDP 18-25 weeks	1270	0.89	98.2%	0.6	0.6	0.6	0.6	4.7
mIDP >25 weeks	1269	0.89	96.7%	0.6	0.6	0.6	0.6	17.8
DNOP								
mCPP <18 weeks	1274	0.008	0.0%	0	0.8	1.4	2.8	120.2
mCPP 18-25 weeks	1270	0.008	0.0%	0	0.5	0.9	1.8	75.4
mCPP >25 weeks	1269	0.008	0.1%	0	0.9	1.8	3.2	60.4

Continue

Continued

	N	LOD	<LOD	min	P25	P50	P75	max
mOP <18 weeks	1274	0.25	90.2%	0.2	0.2	0.2	0.2	9.3
mOP 18-25 weeks	1270	0.25	99.4%	0.2	0.2	0.2	0.2	0.8
mOP >25 weeks	1269	0.25	99.3%	0.2	0.2	0.2	0.2	1.7
mCHpP <18 weeks	1274	0.06	99.3%	0	0	0	0	0.2
mCHpP 18-25 weeks	1270	0.06	100.0%	0	0	0	0	0
mCHpP >25 weeks	1269	0.06	99.8%	0	0	0	0	3.7
Other HMWP								
mBzP <18 weeks	1274	0.15	8.2%	0.1	2.3	5.8	12.1	613.8
mBzP 18-25 weeks	1270	0.15	1.7%	0.1	2.2	5.3	11.0	522.9
mBzP >25 weeks	1269	0.15	3.5%	0.1	1.2	3.1	6.5	211.0
mHxP <18 weeks	1274	0.06	23.9%	0	0.1	0.2	0.5	59.9
mHxP 18-25 weeks	1270	0.06	98.8%	0	0	0	0	0.4
mHxP >25 weeks	1269	0.06	98.1%	0	0	0	0	2.4
mHpP <18 weeks	1274	0.30	35.4%	0.2	0.2	0.6	1.5	78.7
mHpP 18-25 weeks	1270	0.30	96.6%	0.2	0.2	0.2	0.2	1.3
mHpP >25 weeks	1269	0.30	98.5%	0.2	0.2	0.2	0.2	24.5
mCHP <18 weeks	1274	0.04	80.3%	0	0	0	0	22.9
mCHP 18-25 weeks	1270	0.04	94.4%	0	0	0	0	1.4
mCHP >25 weeks	1269	0.04	99.3%	0	0	0	0	2.2
PA								
PA <18 weeks	1274	1.11	0.2%	0.8	30.0	56.9	120.3	9450.0
PA 18-25 weeks	1270	1.11	0.1%	0.8	62.5	157.4	288.6	5367.5
PA >25 weeks	1269	1.11	0.4%	0.8	34.3	69.2	132.0	3580.0

Abbreviations: n=number, LOD=limit of detection, min=minimum, P=percentile, max=maximum. LMWP= Low molecular weight phthalates (Mono methyl phthalate (mMP), Mono ethyl phthalate (mEP), Mono-n-butyl phthalate (mBP), Mono-isobutyl phthalate (mIBP)), HMWP= High molecular weight phthalates (Di-2-ethyl-hexyl phthalates (DEHP) [Mono-(2-ethyl-5-carboxypentyl) phthalate (mECPP), Mono-(2-ethyl-5-hydroxyhexyl) phthalate (mEHHP), Mono-(2-ethyl-5-oxohexyl) phthalate (mEOHP), Mono-[(2-carboxymethyl)hexyl] phthalate (mCMHP)], Di-isononyl phthalate (DINP) [Mono isononyl phthalate (mINP)], Di-isodecylphthalate (DIDP) [Mono-(8-methyl-1-nonyl) phthalate (MIDP)], Di-n-octylphthalate (DNOP) [Mono(3-carboxypropyl) phthalate (mCPP), Monooctyl phthalate (mOP), Mono-(7-carboxy-n-heptyl) phthalate (mCHpP)], other HMWP [Monobenzyl phthalate (mBzP), Mono-hexyl phthalate (mHxP), Mono-2-heptylphthalate (mHpP), Monocyclohexyl phthalate (mCHP)]), PA= Phthalic acid.

a. Values below the LOD are imputed by LOD/√2.

Table S3. Descriptive statistics of urinary bisphenol concentrations in ng/ml [a] across pregnancy measured in 1,282 women

	N	LOD	%<LOD	min	P25	P50	P75	max
Bisphenol A <18 weeks	1274	0.15	20.6%	0.1	0.3	1.1	2.8	524.8
Bisphenol A 18-25 weeks	1270	0.15	6.9%	0.1	0.6	1.3	3.0	365
Bisphenol A >25 weeks	1269	0.15	10.1%	0.1	0.6	1.5	3.0	72.9
Bisphenol S <18 weeks	1274	0.05	32.2%	0	0	0.2	0.6	28.4
Bisphenol S 18-25 weeks	1270	0.05	70.2%	0	0	0	0.1	20.0
Bisphenol S >25 weeks	1269	0.05	81.0%	0	0	0	0	46.7
Bisphenol Z <18 weeks	1274	0.12	88.0%	0.1	0.1	0.1	0.1	2.3
Bisphenol Z 18-25 weeks	1270	0.12	96.2%	0.1	0.1	0.1	0.1	0.4
Bisphenol Z >25 weeks	1269	0.12	99.8%	0.1	0.1	0.1	0.1	1.3
Bisphenol B <18 weeks	1274	0.03	90.2%	0	0	0	0	1.6
Bisphenol B 18-25 weeks	1270	0.03	97.6%	0	0	0	0	0.5
Bisphenol B >25 weeks	1269	0.03	100%	0	0	0	0	0
Bisphenol F <18 weeks	1274	0.18	59.7%	0.1	0.1	0.1	0.4	82.7
Bisphenol F 18-25 weeks	1270	0.18	88.8%	0.1	0.1	0.1	0.1	14.3
Bisphenol F >25 weeks	1269	0.18	71.2%	0.1	0.1	0.1	0.5	42.7
Bisphenol AF <18 weeks	1274	0.79	100%	0.6	0.6	0.6	0.6	0.6
Bisphenol AF 18-25 weeks	1270	0.79	100%	0.6	0.6	0.6	0.6	0.6
Bisphenol AF >25 weeks	1269	0.79	100%	0.6	0.6	0.6	0.6	0.6
Bisphenol AP <18 weeks	1274	0.07	92.4%	0.1	0.1	0.1	0.1	6.3
Bisphenol AP 18-25 weeks	1270	0.07	100%	0.1	0.1	0.1	0.1	0.1
Bisphenol AP >25 weeks	1269	0.07	99.9%	0.1	0.1	0.1	0.1	0.2
Bisphenol P <18 weeks	1274	0.11	98.7%	0.1	0.1	0.1	0.1	0.5
Bisphenol P 18-25 weeks	1270	0.11	100%	0.1	0.1	0.1	0.1	0.1
Bisphenol P >25 weeks	1269	0.11	99.3%	0.1	0.1	0.1	0.1	0.2

Abbreviations: n=number, LOD=limit of detection, min=minimum, P=percentile, max=maximum.
a. Values below the LOD are imputed by LOD/√2.

Table S4. Intra-class correlations between log10 transformed phthalate and bisphenol concentrations across gestational urinary collection phases

	ICC [a]	ICC [b]
Phthalate metabolite concentrations in ng/ml [c]		
LMWP	0.36	0.63
HMWP	0.21	0.44
DEHP	0.19	0.41
DNOP	0.23	0.47
PA	0.20	0.43
phthalate metabolite concentrations in ug/g creatinine		
LMWP	0.27	0.53
HMWP	0.10	0.26
DEHP	0.10	0.25
DNOP	0.12	0.29
PA	0.12	0.29
Bisphenol metabolite concentrations in ng/ml		
Total bisphenols [d]	0.05	0.14
Bisphenol concentrations in ug/g creatinine		
Total bisphenols [d]	0.00	0.00

a. ICC = Intraclass Correlation Coefficients calculated using a single-measurement, absolute-agreement, and 2-way mixed-effects model.
b. ICC = Intraclass Correlation Coefficients calculated using a mean of three measurements, absolute-agreement, and 2-way mixed-effects model.
c. Phthalate metabolites are grouped into: low molecular weight phthalate metabolites (LMWP) = sum of Mono methyl phthalate, Mono ethyl phthalate, Mono-isobutyl phthalate, and Mono-n-butyl phthalate, high molecular weight phthalate metabolites (HMWP) = sum of mono-(2-ethyl-5-carboxypentyl) phthalate, mono-(2-ethyl-5-hydroxyhexyl) phthalate, mono-(2-ethyl-5-ox-ohexyl) phthalate, mono-[(2-carboxymethyl)hexyl] phthalate, mono(3-carboxypropyl) phthalate, monobenzyl phthalate, mono-hexyl phthalate, and mono-2-hep-tylphthalate, Di-2-ethylhexyl phthalate metabolites (DEHP) = sum of Mono-(2-ethyl-5-carboxypentyl) phthalate, Mono-(2-ethyl-5-hydroxyhexyl) phthalate, Mono-(2-ethyl-5-oxohexyl) phthalate, and Mono-[(2-carboxymethyl)hexyl] phthalate, Di-n-octyl phthalate metabolites (DNOP) = Mono(3-carboxypropyl) phthalate, Phthalic acid (PA) = a proxy for total phthalate exposure measured in ug/g creatinine.
d. Total bisphenol =-sum of Bisphenol A, bisphenol F, and Bisphenol S.

Table S5. Adjusted [a] associations between maternal urine phthalate metabolite and bisphenol concentrations in ug/g creatinine, by timing of pregnancy and sex (n=1,282).

	Boys (N=647)		Girls (N=635)			
	B	95%CI	B	95%CI	P-interaction	P-interaction [b]
Phthalate metabolite concentrations [c]						
LMWP <18 weeks	-1.02	-3.05, 1.00	-2.38	-4.24, -0.52	0.34	0.40
LMWP 18-25 weeks	0.05	-2.20, 2.29	0.53	-1.53, 2.59	0.74	0.70
LMWP >25 weeks	-2.28	-4.67, 0.11	-0.25	-2.38, 1.87	0.31	0.19
LMWP Averaged	-2.07	-5.19, 1.06	-1.50	-4.21, 1.20	0.85	0.69
HMWP <18 weeks	-1.94	-4.63, 0.75	-1.68	-4.19, 0.83	0.87	0.82
HMWP 18-25 weeks [d]	1.06	-1.74, 3.86	0.90	-1.53, 3.34	0.73	0.95
HMWP >25 weeks [d]	1.59	-2.28, 5.47	0.19	-3.13, 3.50	0.34	0.57
Averaged HMWP	-0.19	-4.95, 4.58	-0.57	-4.80, 3.66	0.69	0.95
DEHP <18 weeks	-1.95	-4.59, 0.69	-1.55	-4.00, 0.89	0.79	0.78
DEHP 18-25 weeks	0.93	-1.81, 3.66	0.78	-1.61, 3.18	0.79	0.95
DEHP >25 weeks	1.58	-2.14, 5.31	0.05	-3.14, 3.24	0.32	0.51
Averaged DEHP	-0.13	-3.68, 3.41	0.03	-3.03, 3.09	0.95	0.91
DNOP <18 weeks	-1.88	-4.47, 0.70	-2.31	-4.71, 0.08	0.77	0.90
DNOP 18-25 weeks	0.86	-1.99, 3.70	-0.41	-3.16, 2.33	0.44	0.55
DNOP >25 weeks	-2.91	-6.49, 0.68	-1.47	-4.67, 1.74	0.82	0.54
Averaged DNOP	-2.62	-7.06, 1.82	-3.63	-7.90, 0.63	0.55	0.81
PA <18 weeks	-1.92	-4.35, 0.51	-1.91	-4.09, 0.27	0.81	0.86
PA 18-25 weeks	2.17	-0.35, 4.68	-0.26	-2.64, 2.12	0.15	0.16
PA >25 weeks	-2.19	-5.34, 0.96	0.07	-2.90, 3.04	0.32	0.28
Averaged PA	-1.15	-5.26, 2.95	-2.15	-6.01, 1.70	0.79	0.80
Bisphenol concentrations						
TB <18 weeks [e]	0.95	-1.15, 3.05	-0.15	-1.99, 1.70	0.68	0.45
TB 18-25 weeks [f]	0.56	-1.68, 2.81	-0.25	-2.48, 1.99	0.54	0.56
TB >25 weeks [g]	1.36	-1.06, 3.78	-1.72	-4.06, 0.63	0.08	0.07
Averaged TB	2.83	-1.06, 6.72	-1.81	-5.48, 1.85	0.13	0.08

a. Adjusted for maternal age, maternal non-verbal IQ, age of the child at assessment, ethnicity categories (Dutch, other-western and non-western), education (low, intermediate and high), income (low, middle and high), marital status, maternal alcohol consumption (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasionally alcohol consumption during pregnancy and frequently alcohol consumption during pregnancy), BMI, parity categories (0, 1, 2+), and smoking categories (no smoking during pregnancy, smoked until pregnancy was known, smoked during pregnancy).

b. Estimated with the use of the augmented product terms (Buckley et al. 2017).

c Phthalate metabolites are grouped into: low molecular weight phthalate metabolites (LMWP) = sum of Mono methyl phthalate, Mono ethyl phthalate, Mono-isobutyl phthalate, and Mono-n-butyl phthalate, high molecular weight phthalate metabolites (HMWP) = sum of mono-(2-ethyl-5-carboxypentyl) phthalate, mono-(2-ethyl-5-hydroxyhexyl) phthalate, mono-(2-ethyl-5-ox-ohexyl) phthalate, mono-[(2-carboxymethyl)hexyl] phthalate, mono(3-carboxypropyl) phthalate, monobenzyl phthalate, mono-hexyl phthalate, and mono-2-hep-tylphthalate, Di-2-ethylhexyl phthalate metabolites (DEHP) = sum of Mono-(2-ethyl-5-carboxypentyl) phthalate, Mono-(2-ethyl-5-hydroxyhexyl) phthalate, Mono-(2-ethyl-5-oxohexyl) phthalate, and Mono-[(2-carboxymethyl)hexyl] phthalate, Di-n-octyl phthalate metabolites (DNOP) = Mono(3-carboxypropyl) phthalate, Phthalic acid (PA) = a proxy for total phthalate exposure measured in ug/g creatinine.

d. with the exclusion of mono-hexyl phthalate, and mono-2-hep-tyl phthalate metabolite concentrations

e. Total bisphenol =-sum of Bisphenol A, bisphenol F, and Bisphenol S.

f. Total bisphenol =-sum of Bisphenol A, and Bisphenol S.

g. Total bisphenol =-sum of Bisphenol A and bisphenol F.

Table S6. Difference in child non-verbal IQ score at age six years per log10 increase in maternal urinary phthalate and bisphenol concentrations in ng/ml with creatinine added as a separate covariate (n=1,282).

		Adjusted [a]	
Phthalate metabolites [b]		B	95%CI
Σ LMWP metabolites			
	<18 weeks	-1.65	-3.02, -0.27
	18-25 weeks	0.16	-1.37, 1.69
	>25 weeks	-1.31	-2.86, 0.24
	Averaged	-1.82	-3.83, 0.19
Σ HMWP metabolites			
	<18 weeks	-1.92	-3.88, 0.04
	18-25 weeks [c]	0.95	-0.98, 2.88
	>25 weeks [c]	0.01	-2.48, 2.50
	Averaged	-0.86	-4.08, 2.35
Σ DEHP metabolites			
	<18 weeks	-1.80	-3.69, 0.09
	18-25 weeks	0.82	-1.05, 2.70
	>25 weeks	-0.02	-2.40, 2.36
	Averaged	-0.91	-4.00, 2.19
Σ DNOP metabolites			
	<18 weeks	-2.03	-3.86, -0.21
	18-25 weeks	0.28	-1.66, 2.22
	>25 weeks	-2.82	-5.10, -0.54
	Averaged	-3.32	-6.31, -0.34
PA metabolites			
	<18 weeks	-1.99	-3.68, -0.30
	18-25 weeks	0.79	-1.05, 2.64
	>25 weeks	-1.48	-3.54, 0.57
	Averaged	-2.20	-5.03, 0.64
		Adjusted [a]	
Bisphenol metabolites		B	95%CI
	<18 weeks [d]	0.65	-0.85, 2.15
	18-25 weeks [e]	0.02	-1.56, 1.59
	>25 weeks [f]	-0.28	-2.00, 1.44
	Averaged	0.47	-2.27, 3.21

a. Adjusted for creatinine, maternal age, maternal non-verbal IQ, sex of child, age of the child at assessment, ethnicity, education, income, marital status, maternal alcohol consumption, BMI, parity categories, and smoking.
b Phthalate metabolites are grouped into: low molecular weight phthalate metabolites (LMWP) = sum of Mono methyl phthalate, Mono ethyl phthalate, Mono-isobutyl phthalate, and Mono-n-butyl phthalate, high molecular weight phthalate metabolites (HMWP) = sum of mono-(2-ethyl-5-carboxypentyl) phthalate, mono-(2-ethyl-5-hydroxyhexyl) phthalate, mono-(2-ethyl-5-ox-ohexyl) phthalate, mono-[(2-carboxymethyl)hexyl] phthalate, mono(3-carboxypropyl) phthalate, monobenzyl phthalate, mono-hexyl phthalate, and mono-2-hep-tylphthalate, Di-2-ethylhexyl phthalate metabolites (DEHP) = sum of Mono-(2-ethyl-5-carboxypentyl) phthalate, Mono-(2-ethyl-5-hydroxyhexyl) phthalate, Mono-(2-ethyl-5-oxohexyl) phthalate, and Mono-[(2-carboxymethyl)hexyl] phthalate, Di-n-octyl phthalate metabolites (DNOP) = Mono(3-carboxypropyl) phthalate, Phthalic acid (PA) = a proxy for total phthalate exposure measured in ug/g creatinine.
c. with the exclusion of mono-hexyl phthalate, and mono-2-hep-tyl phthalate metabolite concentrations
d Total bisphenol =-sum of Bisphenol A, bisphenol F, and Bisphenol S.
e Total bisphenol =-sum of Bisphenol A, and Bisphenol S.
f. Total bisphenol =-sum of Bisphenol A and bisphenol F.

Table S7. Difference in child non-verbal IQ score at age six years per log10 increase in individual urinary biomarkers (ug/g creatinine) of phthalate and bisphenol exposure (n=1,282).

	<18 weeks		18-25 weeks		>25 weeks		Averaged	
Unadjusted	B	95%CI	B	95%CI	B	95%CI	B	95%CI
mMP	-2.70	-4.42, -0.99	-0.90	-2.75, 0.94	-0.43	-2.26, 1.40	-2.80	-5.33, -0.27
mEP	-2.26	-3.42, -1.09	-1.06	-2.34, 0.23	-1.90	-3.23, -0.56	-3.02	-4.66, -1.39
mIBP	-3.85	-5.38, -2.31	-2.35	-4.06, -0.64	-2.85	-4.68, -1.01	-6.11	-8.46, -3.75
mBP	-2.31	-3.89, -0.72	-1.35	-3.28, 0.58	-1.77	-3.73, 0.18	-4.26	-6.96, -1.56
mECPP	-3.30	-5.14, -1.47	-1.61	-3.54, 0.32	-0.66	-3.19, 1.87	-4.53	-7.52, -1.54
mEHHP	-2.22	-3.86, -0.58	0.10	-1.72, 1.93	0.71	-1.23, 2.66	-1.59	-4.41, 1.22
mEOHP	-2.05	-3.65, -0.46	-0.63	-2.33, 1.08	0.41	-1.65, 2.47	-2.49	-5.33, 0.36
mCMHP	-3.74	-5.71, -1.77	0.31	-1.63, 2.24	0.67	-1.54, 2.87	-2.51	-5.70, 0.68
mCPP	-2.61	-4.46, -0.76	-0.13	-2.20, 1.94	-1.10	-3.64, 1.43	-3.28	-6.47, -0.09
mBzBP	-2.52	-3.74, -1.30	-1.49	-2.96, -0.03	-1.27	-2.81, 0.26	-4.07	-6.10, -2.04
mHxP	0.69	-0.68, 2.07	-	-	-	-	-	-
mHpP	-2.29	-3.84, -0.73	-	-	-	-	-	-
PA	-2.59	-4.32, -0.87	-0.90	-2.72, 0.91	-2.46	-4.75, -0.17	-4.64	-7.57, -1.71
BPA	-0.44	-1.69, 0.82	0.59	-0.99, 2.18	0.25	-1.38, 1.88	-0.44	-1.69, 0.82
BPS	0.99	-0.08, 2.06	0.10	-1.57, 1.76	-	-	1.43	-0.35, 3.22
PBF	2.34	0.85, 3.82	-	-	2.10	0.71, 3.50	4.36	2.35, 6.36
	<18 weeks		18-25 weeks		>25 weeks		Averaged	
Adjusted [a]	B	95%CI	B	95%CI	B	95%CI	B	95%CI
mMP	-1.56	-3.17, 0.05	-0.08	-1.81, 1.65	-0.67	-2.39, 1.06	-1.62	-4.00, 0.76
mEP	-1.30	-2.41, -0.19	0.17	-1.05, 1.39	-0.97	-2.24, 0.29	-1.28	-2.85, 0.29
mIBP	-1.47	-2.97, 0.04	0.49	-1.17, 2.14	0.48	-1.31, 2.27	-0.61	-3.00, 1.78
mBP	-1.03	-2.53, 0.47	1.05	-0.80, 2.90	-0.01	-1.87, 1.86	-0.34	-2.96, 2.28
mECPP	-1.74	-3.48, 0.00	0.12	-1.70, 1.95	0.05	-2.33, 2.42	-1.43	-4.30, 1.43
mEHHP	-1.32	-2.87, 0.22	0.87	-0.85, 2.58	0.64	-1.20, 2.48	-0.16	-2.83, 2.52
mEOHP	-1.23	-2.73, 0.28	0.21	-1.39, 1.82	0.85	-1.09, 2.79	-0.57	-3.27, 2.14
mCMHP	-1.98	-3.84, -0.12	1.44	-0.37, 3.25	0.72	-1.35, 2.79	0.11	-2.91, 3.12
mCPP	-1.98	-3.72, -0.23	0.38	-1.57, 2.32	-2.39	-4.76, -0.02	-2.97	-6.00, 0.06
mBzBP	-0.67	-1.84, 0.50	0.97	-0.46, 2.39	0.21	-1.24, 1.66	0.13	-1.88, 2.13
mHxP	0.55	-0.74, 1.83	-	-	-	-	-	-
mHpP	-0.20	-1.70, 1.29	-	-	-	-	-	-
PA	-1.93	-3.55, -0.31	0.85	-0.87, 2.57	-1.09	-3.25, 1.07	-1.77	-4.57, 1.04
BPA	0.22	-0.95, 1.40	0.26	-1.22, 1.75	-0.18	-1.70, 1.34	0.22	-0.95, 1.40
BPS	0.50	-0.50, 1.50	-0.54	-2.09, 1.01	-	-	0.39	-1.29, 2.06
PBF	1.25	-0.15, 2.65	-	-	0.64	-0.68, 1.96	1.85	-0.07, 3.78

Abbreviations: mMP= mono-methyl phthalate, mEP= mono-ethyl phthalate, mIBP= mono-isobutyl phthalate, mBP= mono-n-butyl phthalate, mECPP= mono- (2-ethyl-5-carboxypentyl) phthalate, mEHHP= mono- (2-ethyl-5-hydroxyhexyl) phthalate, mEOHP= mono-(2-ethyl-5-oxohexyl) phthalate, mCMHP= mono-[(2-carboxymethyl)hexyl] phthalate, mCPP= mono(3-carboxypropyl) phthalate, mBzP= monobenzyl phthalate, mHxP= mono-hexyl phthalate, mHpP= mono-2-heptyl phthalate, PA= phthalic acid, BPA= bisphenol A, BPS= bisphenol S, BPF= bisphenol F.

a. Adjusted for maternal age, maternal non-verbal IQ, sex of child, age of the child at assessment, ethnicity, education, income, marital status, maternal alcohol consumption, BMI, parity categories, and smoking.

Table S8. inverse probability weighted association between log10 transformed maternal urine phthalate and bisphenol metabolite concentrations in ug/g creatinine and child non-verbal IQ score at age six years (n=1,282).

		Adjusted	
Phthalate metabolites [c]		B	95%CI
Σ LMWP metabolites			
	<18 weeks	-1.70	-3.06, -0.34
	18-25 weeks	0.25	-1.27, 1.76
	>25 weeks	-1.15	-2.73, 0.42
	Averaged	-1.74	-3.75, 0.28
Σ HMWP metabolites			
	<18 weeks	-1.53	-3.36, 0.29
	18-25 weeks [e]	1.23	-0.63, 3.08
	>25 weeks [e]	0.53	-1.98, 3.05
	Averaged	-0.05	-3.23, 3.12
Σ DEHP metabolites			
	<18 weeks	-1.49	-3.27, 0.30
	18-25 weeks	1.14	-0.67, 2.95
	>25 weeks	0.49	-1.93, 2.92
	Averaged	0.41	-1.92, 2.75
Σ DNOP metabolites			
	<18 weeks	-2.00	-3.73, -0.27
	18-25 weeks	0.28	-1.66, 2.22
	>25 weeks	-2.81	-5.16, -0.46
	Averaged	-3.38	-6.41, -0.34
PA metabolites			
	<18 weeks	-1.93	-3.53, -0.32
	18-25 weeks	1.02	-0.70, 2.75
	>25 weeks	-1.33	-3.47, 0.81
	Averaged	-1.77	-4.56, 1.02
		Adjusted	
Bisphenol metabolites		B	95%CI
	<18 weeks [f]	0.72	-0.66, 2.11
	18-25 weeks [g]	0.22	-1.35, 1.79
	>25 weeks [h]	-0.04	-1.72, 1.64
	Averaged	1.07	-1.59, 3.73

a. Adjusted for maternal age, maternal non-verbal IQ, sex of child, age of the child at assessment, ethnicity, education, income, marital status, maternal alcohol consumption, BMI, parity categories, and smoking.
b. Adjusted model with the inclusion of the three exposures in one model.
c Phthalate metabolites are grouped into: low molecular weight phthalate metabolites (LMWP) = sum of Mono methyl phthalate, Mono ethyl phthalate, Mono-isobutyl phthalate, and Mono-n-butyl phthalate, high molecular weight phthalate metabolites (HMWP) = sum of mono-(2-ethyl-5-carboxypentyl) phthalate, mono-(2-ethyl-5-hydroxyhexyl) phthalate, mono-(2-ethyl-5-ox-ohexyl) phthalate, mono-[(2-carboxymethyl)hexyl] phthalate, mono(3-carboxypropyl) phthalate, monobenzyl phthalate, mono-hexyl phthalate, and mono-2-hep-tylphthalate, Di-2-ethylhexyl phthalate metabolites (DEHP) = sum of Mono-(2-ethyl-5-carboxypentyl) phthalate, Mono-(2-ethyl-5-hydroxyhexyl) phthalate, Mono-(2-ethyl-5-oxohexyl) phthalate, and Mono-[(2-carboxymethyl)hexyl] phthalate, Di-n-octyl phthalate metabolites (DNOP) = Mono(3-carboxypropyl) phthalate, Phthalic acid (PA) = a proxy for total phthalate exposure measured in ug/g creatinine.
d. Tests whether exposure from different time points relates in the same manner to non-verbal IQ scores using the multiple informant method (Sanchez et al. 2011).
e. with the exclusion of mono-hexyl phthalate, and mono-2-hep-tyl phthalate metabolite concentrations
f. Total bisphenol =-sum of Bisphenol A, bisphenol F, and Bisphenol S.
g Total bisphenol =-sum of Bisphenol A, and Bisphenol S.
h. Total bisphenol =-sum of Bisphenol A and bisphenol F.

Table S9. Adjusted [a] association between log10 transformed maternal urinary phthalate metabolite and bisphenol concentration in ug/g creatinine with additional adjustment for the maternal intake of fruit [b], vegetables [b] and folic acid (n=1,282).

		B	95%CI
Phthalate metabolites [c]			
Σ LMWP			
	<18 weeks	-1.64	-3.01, -0.28
	18-25 weeks	0.29	-1.23, 1.81
	>25 weeks	-1.05	-2.64, 0.54
	Averaged	-1.63	-3.67, 0.41
Σ HMWP			
	<18 weeks	-1.72	-3.55, 0.11
	18-25 weeks [d]	1.04	-0.80, 2.88
	>25 weeks [d]	0.65	-1.88, 3.18
	Averaged	-0.33	-3.49, 2.82
Σ DEHP			
	<18 weeks	-1.66	-3.45, 0.13
	18-25 weeks	0.91	-0.89, 2.71
	>25 weeks	0.59	-1.85, 3.02
	Averaged	0.08	-2.23, 2.39
Σ DNOP			
	<18 weeks	-1.93	-3.68, -0.18
	18-25 weeks	0.40	-1.55, 2.34
	>25 weeks	-2.41	-4.79, -0.03
	Averaged	-2.92	-5.95, 0.12
PA			
	<18 weeks	-1.85	-3.47, -0.22
	18-25 weeks	0.84	-0.88, 2.56
	>25 weeks	-1.01	-3.18, 1.16
	Averaged	-1.65	-4.45, 1.16
Bisphenol concentrations			
	<18 weeks [e]	0.51	-0.87, 1.90
	18-25 weeks [f]	0.16	-1.40, 1.73
	>25 weeks [g]	0.00	-1.68, 1.68
	Averaged	0.79	-1.87, 3.46

a. Adjusted for maternal age, maternal non-verbal IQ, sex of child, age of the child at assessment, ethnicity, maternal education, income, marital status, maternal alcohol consumption, maternal BMI, parity categories, maternal smoking, maternal fruit intake, maternal vegetable intake, and maternal folic acid intake.
b. maternal fruit and vegetable intake were adjusted for energy intake.
c Phthalate metabolites are grouped into: low molecular weight phthalate metabolites (LMWP) = sum of Mono methyl phthalate, Mono ethyl phthalate, Mono-isobutyl phthalate, and Mono-n-butyl phthalate, high molecular weight phthalate metabolites (HMWP) = sum of mono-(2-ethyl-5-carboxypentyl) phthalate, mono-(2-ethyl-5-hydroxyhexyl) phthalate, mono-(2-ethyl-5-ox-ohexyl) phthalate, mono-[(2-carboxymethyl)hexyl] phthalate, mono(3-carboxypropyl) phthalate, monobenzyl phthalate, mono-hexyl phthalate, and mono-2-hep-tylphthalate, Di-2-ethylhexyl phthalate metabolites (DEHP) = sum of Mono-(2-ethyl-5-carboxypentyl) phthalate, Mono-(2-ethyl-5-hydroxyhexyl) phthalate, Mono-(2-ethyl-5-oxohexyl) phthalate, and Mono-[(2-carboxymethyl)hexyl] phthalate, Di-n-octyl phthalate metabolites (DNOP) = Mono(3-carboxypropyl) phthalate, Phthalic acid (PA) = a proxy for total phthalate exposure measured in ug/g creatinine.
d. with the exclusion of mono-hexyl phthalate, and mono-2-hep-tyl phthalate metabolite concentrations
e. Total bisphenol =-sum of Bisphenol A, bisphenol F, and Bisphenol S.
f. Total bisphenol =-sum of Bisphenol A, and Bisphenol S.
g. Total bisphenol =-sum of Bisphenol A and bisphenol F.

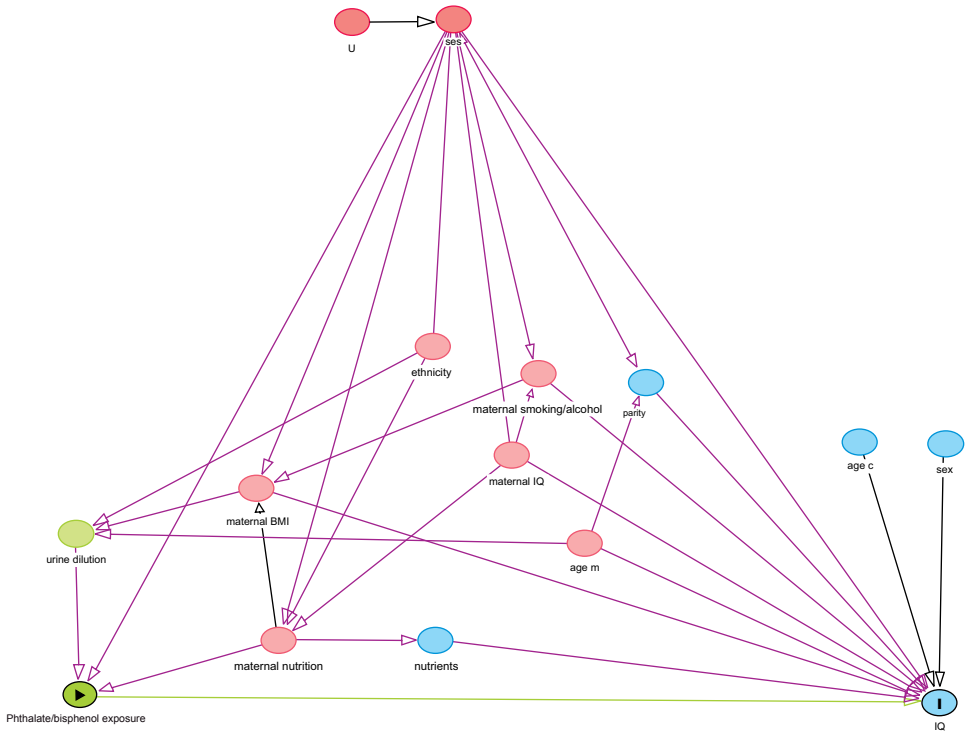

Figure S1. Directed Acyclic Graph of the prenatal phthalates and bisphenol exposure and child non-verbal IQ association.

Potential adjustment variables were selected a priori defined with a Directed Acyclic Graph (DAG) using the Dagitty software (Textor et al. 2017). The DAG was based on previous studies of phthalates and bisphenol exposure and child neurodevelopment and on biologically plausible covariate–exposure and covariate–outcome associations observed in our data. Green circles represent ancestors of the exposure, blue circles ancestors of the outcome, pink circles ancestors of both exposure and outcome. **Maternal BMI**= Maternal body mass index, **ses**= socioeconomic status (maternal education, household income and marital status), age c= child age at assessment, age m= age mother, IQ= nonverbal intelligent quotient, U=unobserved ancestor of socioeconomic status.

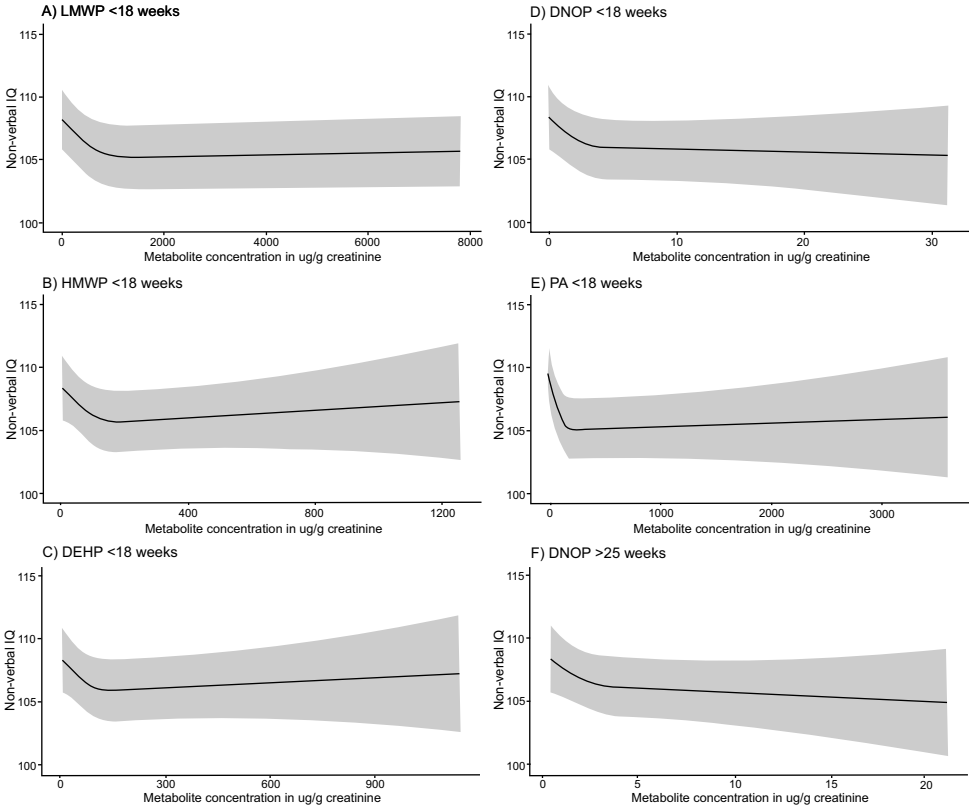

Figure S2. Restricted cubic splines (and 95% confidence intervals) of adjusted child non-verbal IQ scores and (untransformed) grouped phthalate metabolite concentrations. A) LMWP= Low molecular weight phthalates (sum of mono methyl phthalate, mono ethyl phthalate, mono-n-butyl phthalate, mono-isobutyl phthalate) metabolites measured at <18 weeks of gestation. B) HMWP= High molecular weight phthalates (sum of mono-(2-ethyl-5-carboxypentyl) phthalate (mECPP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (mEHHP), mono(2-ethyl-5-oxohexyl) phthalate (mEOHP), mono-[(2-carboxymethyl) hexyl] phthalate (mCMHP), Mono(3-carboxypropyl) phthalate (mCPP), monobenzyl phthalate, mono-hexyl phthalate, Mono-2-heptyl phthalate) metabolites measured at <18 weeks of gestation. **C)** DEHP= Di-2-ethylhexyl phthalates (sum of mECPP, mEHHP, mEOHP, and mCMHP) metabolites measured at <18 weeks of gestation**. D)** DNOP= Di-n-octyl phthalate (mCPP) metabolites measured at <18 weeks of gestation. **E)** PA=phthalic acid measured at <18 weeks of gestation. **F)** DNOP metabolites measured at >25 weeks of gestation.

4

Chapter 4

Organophosphate pesticide metabolite concentrations in urine during pregnancy and offspring nonverbal IQ at age 6 years

Jusko, T. A., van den Dries, M. A., Pronk, A., Shaw, P. A., Guxens, M., Spaan, S., Jaddoe, V.W.V., Tiemeier, H., & Longnecker, M. P. (2019).

Environmental health perspectives, 127(1), 17007.

Abstract

Background: Susceptibility to organophosphate (OP) pesticide neurotoxicity may be greatest during the prenatal period; however, previous studies have produced mixed findings concerning in utero OP pesticide exposure and child cognition.

Objectives: Our objective was to determine whether maternal urinary concentrations of OP pesticide metabolites are inversely associated with child nonverbal IQ at 6 y of age and to examine potential effect measure modification by the PON1 gene.

Methods: Data came from 708 mother–child pairs participating in the Generation R Study. Maternal urine concentrations of six dialkylphosphates (DAPs), collected at <18, 18–25, and >25 weeks of gestation, were determined. Child nonverbal IQ was measured at 6 y of age using the Mosaics and Categories subtests from the Snijders-Oomen Nonverbal Intelligence Test–Revised. PON1 was determined in cord blood for 474 infants. Multiple linear regression models were fit to estimate the DAP–IQ associations and PON1 interactions.

Results: Overall, associations between child nonverbal IQ and maternal DAP concentrations were small and imprecise, and these associations were inconsistent across urine sampling periods. However, for a 10-fold difference in total DAP concentration for the >25 weeks of gestation samples, adjusted child nonverbal IQ was 3.9 points lower (95% CI: −7.5, −0.3). Heterogeneity in the DAP–IQ association by PON1 gene allele status was not observed (n=474).

Conclusions: Consistent evidence of an association between higher maternal urinary DAP concentrations and lower child IQ scores at 6 y of age was not observed. There was some evidence for an inverse relation of child nonverbal IQ and late pregnancy urinary DAPs, but the estimated association was imprecise.

Introduction

Organophosphate (OP) pesticides have been used for more than 50 y because they enhance crop yield and degrade rapidly. Some of the active pesticide, however, stays on food crops, and metabolites are often detected in human consumers.[1] Most exposure in the general population is from diet,[2-4] though other exposure routes can be important in selected populations.[5] OP pesticide toxicity at high doses, via inhibition of acetylcholinesterase, has been well described;[6] whether toxicity at lower doses occurs, via other mechanisms,[7,8] is unclear. Because susceptibility to adverse effects on cognition may be greatest during early development, many studies of low-dose exposure have focused on prenatal exposure, and the results have been suggestive in some cases[9-12] but inconclusive overall.[13] This heterogeneity may be explained by underlying genetic factors, specifically the PON1 gene, which may modify the association between organophosphate pesticide exposure and cognition.[13,14] Although the use of OP pesticides has been reduced, recent data show that the levels of metabolites in population biomonitoring studies have been stable for at least the first decade of this millennium.[15]

Among the dozens of OP pesticides in current use, some yield specific metabolites; most, however, degrade to one or more dialkylphosphates (DAPs), and the measurement of six DAPs in urine is the most-used method of estimating exposure to this class of compounds.[16] The concentration of DAPs in urine reflects exposure in the past day or two, and individual exposure varies substantially from day to day, depending on diet.[3] This intraindividual variability means that estimates of exposure are improved if urine specimens are collected from an individual at more than one point in time. Often, however, epidemiologic studies have had at most two urine specimens per subject, which may have limited their statistical power to detect adverse associations.[17]

The present study examines maternal urinary concentrations of OP pesticide metabolites in relation to child nonverbal IQ at 6 y of age, with potential effect measure modification by the PON1 gene. Maternal urinary concentrations of OP pesticide metabolites were measured at three time points during pregnancy in the present study, with the intent to reduce possible exposure misclassification.

Methods

Study population and follow-up

Generation R is a prospective population-based birth cohort designed to identify early environmental and genetic determinants of development throughout life and has been described in detail previously.[18] Briefly, all mothers who resided in the study area in

Rotterdam, Netherlands, and had a delivery date between April 2002 and January 2006 were eligible. Mothers were enrolled during pregnancy or in the first months after the birth of their child when newborns visited the routine child health centers. Among the 9,778 mothers who participated in the study, 8,879 (91%) were enrolled during pregnancy (Figure 1). Among the 4,918 women enrolled during pregnancy between February 2004 and January 2006, spot urine specimens during early, middle, and late pregnancy (<18, 18–25, >25 weeks of gestational age, respectively) were collected at the time of routine ultrasound examinations, which occurred throughout the day. In total, 2,083 women provided a complete set of three urine specimens. Mothers provided written informed consent for themselves and their children at the time of enrollment. The study protocol underwent human subjects review at Erasmus Medical Center, Rotterdam, Netherlands (institutional review board registration no. IRB00001482).

When the child was 6 y of age, families were invited to participate in an in-person follow-up to collect cognitive data, additional biospecimens, and sociodemographic and health data. Of the 2,083 mother–child pairs with three pregnancy urine specimens, 1,998 (96%) were followed to 6 y of age. From these 1,998 mother–child pairs with three prenatal urine specimens, women with missing data on maternal age and children with missing data on sex, birth weight, and without Child Behavior Checklist (CBCL) data at 6 y of age were excluded (Figure 1). This resulted in a total of 1,449 mother–child pairs. From these 1,449 pairs, 800 mothers were selected using a random number generator for analyses of DAP metabolites in the maternal urine samples. Given our assumptions, 800 mothers was sufficient to provide 80% power to detect a 2-point decrement in IQ per loge unit increase in average DAP concentration (calculations not shown). Next, 16 participants were excluded from the lab analyses due to insufficient urine specimens. The final analytic sample included 708 mother–child pairs with exposure and outcome data and who had a sufficient volume of urine for analysis.

Urine collection and analysis of DAP metabolites

Details of maternal and 6-y-old child urine specimen collection have been described elsewhere[19] All urine samples were collected between 0800 and 2000 hours in 100-mL polypropylene urine collection containers that were kept for a maximum of 20 h in a cold room (4°C) before being frozen at −20°C in 20-mL portions in 25-mL polypropylene vials. Measurements of six nonspecific DAP metabolites of OP pesticides were conducted at the Institut National de Santé Publique (INSPQ) in Quebec, Canada, using gas chromatography coupled with tandem mass spectrometry (GC–MS/MS).[20] Three dimethyl (DM) metabolites [dimethylphosphate (DMP), dimethylthiophosphate (DMTP), and dimethyldithiophosphate (DMDTP)] were determined, as well as three diethyl (DE) metabolites [diethylphosphate (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP)]. The limit of detection (LOD) was 0.26μg/L for

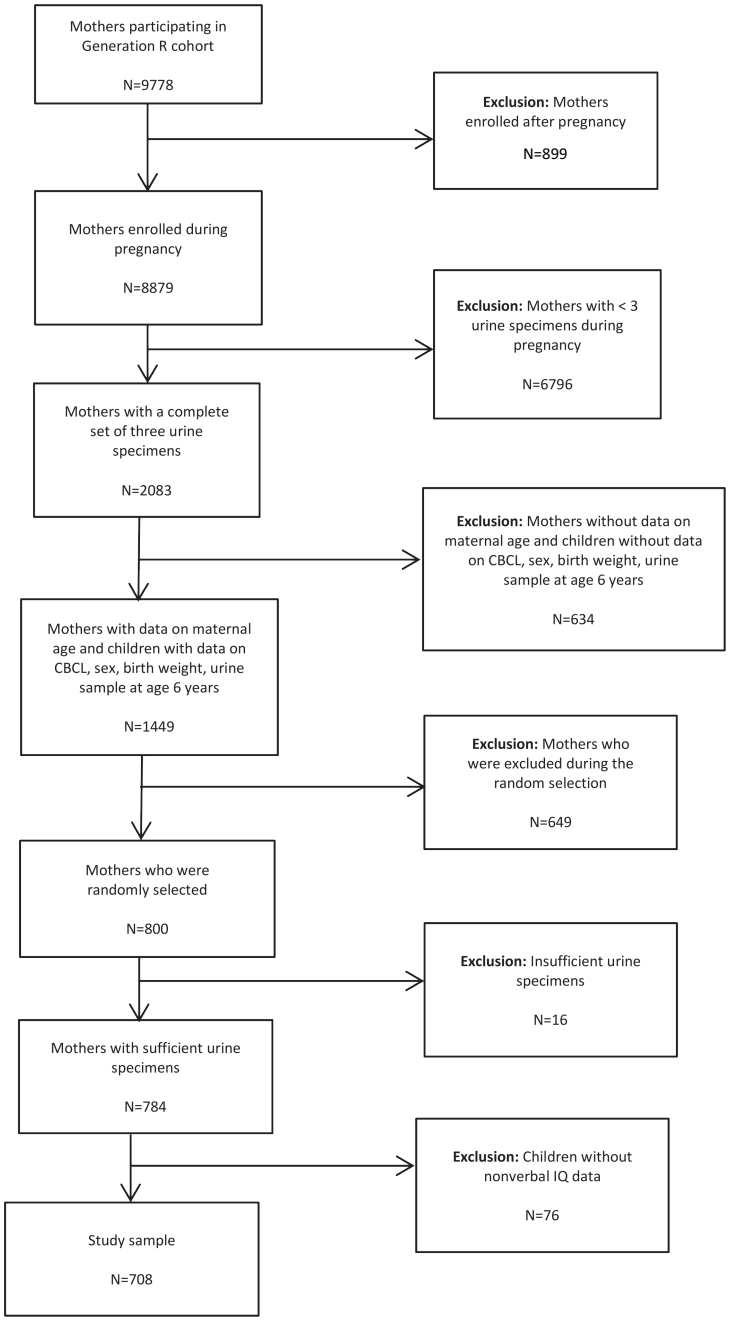

Figure 1. Study sample selection (n=708) from the overall Generation R cohort (n=9,778). Note: CBCL, Child Behavior Checklist.

DMP, 0.40 for DMTP, 0.09 for DMDTP, 0.50 for DEP, 0.12 for DETP, and 0.06 for DEDTP. Measured values below the LOD were included in the data analysis. The inter-day precision of the method during this project, expressed as the coefficient of variation percent, varied between 4.2 and 8.8 for DEDTP, 4.1 and 7.2 for DEP, 5.0 and 9.1 for DETP, 5.5 and 7.1 for DMDTP, 5.3 and 8.0 for DMP, and 5.5 and 7.7 for DMTP based on reference materials (clinical check-urine level II 637 E-495 and MRM E-459).

Molar concentrations were used to facilitate comparison of our results with those from other studies, based on the following molecular weights: DMP 126.0, DMTP 142.1, DMDTP 158.2, DEP 154.1, DETP 170.2, and DEDTP 186.2g/mol. To account for urinary dilution, creatinine concentrations were determined based on the Jaffe reaction.[21,22] The limit of detection for creatinine was 0.28 mmol/L, and the day-to-day coefficient of variation percent varied between 3.0 and 3.3.

Assessment of child nonverbal IQ at 6 y of age

The children's nonverbal IQ was assessed by administering the Mosaics and Categories subtests from the Snijders-Oomen Nonverbal Intelligence Test–Revised, a well-validated instrument developed in Netherlands.[23] These two language-independent subtests include items that probe visuospatial and abstract reasoning abilities and were selected because of the multiethnic composition of the Generation R Study. Raw scores were derived for each subtest and standardized to reflect a mean and standard deviation of the Dutch normative population for ages 2.5–7y. The sum of the standardized scores of the two subtests was converted into the SON-R IQ score using age-specific reference scores provided in the SON-R 2½-7 manual (mean=100, standard deviation=15). These standardized scores, based on the two subtests, correlated well (r=0.86) with those based on the complete instrument.[24]

Genetic analyses

In total, 474 children in the study sample had genetic data available from cord blood. Genotyping was performed using Illumina 610K and 660W arrays. Quality control included filters for sample (≥97.5%) and single nucleotide polymorphism (SNP) call rates (≥95%), minor allele frequencies (MAF; ≥1%), and deviations from the Hardy-Weinberg equilibrium (p≤10–7). We additionally checked heterozygosity, sex accuracy, and relatedness. From this data set we extracted information on rs705379 (PON1-108), rs705381 (PON161), rs854560 (PON1-L55M), rs854572 (PON1-909), and rs662 (PON1-Q192). Only the latter was directly genotyped, all others were imputed. We used Mach 1.0[25] to impute to the 1000 Genomes Iv3 reference panel.[26] All four imputed SNPs had excellent imputation quality (all R2>0.95) and high MAF (all MAF>26%). SNPs were included as allele dosages ranging from zero to two copies of the effects allele.[27] See Table S1 for effect allele and SNP descriptive statistics.

Additional data collection

Maternal reproductive, sociodemographic, and cognitive data were assessed by multiple questionnaires and instruments throughout the study. During pregnancy, data on maternal height and weight were collected as was information on maternal age, parity (0, 1, or ≥2), smoking (no smoking during pregnancy, smoked until pregnancy was recognized, or continued smoking during pregnancy), alcohol intake during pregnancy [no alcohol consumption during pregnancy, alcohol consumption until pregnancy was recognized, continued occasionally (<1 glass/week), or continued frequently (≥1 glass/week)], marital status (married/partner or single), household total net income [<1,200 euros per month (i.e., below the Dutch social security level), 1,200–2,000 euros per month, >2,000 euros per month, highest completed education level (low: <3y at general secondary school; intermediate: ≥3y of secondary education; or high: university degree or higher vocational training), and ethnicity (Dutch national origin, other-Western, or non-Western)]. In addition, maternal dietary intake in the first trimester was assessed using a modified version of a validated semiquantitative food frequency questionnaire (FFQ), and the 293 food items were reduced to 24 predefined food groups (e.g., meat, grains, vegetables, fruits) according to the European Prospective Investigation into Cancer and Nutrition (EPIC)–soft classification, based on origin, culinary usage, and nutrient profiles.[4,28]

An adapted Infant/Toddler Home Observation for Measurement of the Environment (IT-HOME) inventory[29] was administered during a home visit at approximately 3 months of age (SD=1.17 months). The validated 29-item version of the IT-HOME was used to measure the events, objects, and social interactions experienced by the child in the family context.[30] Higher scores on the IT-HOME indicate a more enriched environment.

Maternal nonverbal IQ was measured when mother–child pairs attended the 6-y examination, and was assessed using a computerized Raven's Advanced Progressive Matrices Test, set I[31] The test is a 12-item reliable and validated short version of the Raven's Progressive Matrices to assess nonverbal cognitive ability.[32]

Statistical methods

Exposure

The three DM metabolites (DMP, DMTP, and DMDTP) were summed as total DM (nmol/L) and the three DE metabolites (DEP, DETP, and DEDTP) were summed as total DE (nmol/L). Total DAP concentrations (nmol/L) were calculated by summing the six metabolites. Urinary concentrations were expressed on a volume and creatinine basis (nmol/g creatinine). Missing DAP metabolite values and missing covariate data were imputed (10 times) with the Multivariate Imputation by Chained Equation (MICE) method

in R (version 3.2.3; R Development Core Team).[33,34] DAP metabolite concentrations were log10 transformed before running the multiple imputation (MI) procedure. Child nonverbal IQ was included as a predictor but was not imputed. Apart from household income (13%) and the IT-HOME score (29%), the percentage of missing values did not exceed 10% before imputation.

Statistical model

Initial exploratory data analyses suggested that the OP pesticide–nonverbal IQ associations were nonlinear in functional form and that multivariable models with log10-transformed exposure generally provided a better model fit than models with OP pesticide concentration untransformed (see Table S2). Thus, first, parametric models with DAP concentrations log10-transformed were fit to estimate the OP pesticide–nonverbal child IQ associations, and second, restricted cubic spline models were fit to describe the functional form of the exposure–outcome association utilizing the rms-package in R, which estimates a p-value for nonlinearity (i.e., statistically significant values indicate a departure from linearity) (Harrell 2018). These associations were graphically depicted by plots. In parametric models, nonverbal IQ associations for each urine collection phase (gestational age <18, 18–25, and >25 weeks) were modeled a) separately, b) as a single average of prenatal exposure, and c) for each urine collection phase modeled jointly (i.e., all three DAP concentrations included as three separate terms in each model). The first two regression models (model for each urine collection phase separately and the model for average prenatal exposure) consisted of an unadjusted model and an adjusted model. The third model consisted of a mutually adjusted model in which the three exposures from each time period were jointly estimated. The adjustment variables were maternal age, ethnicity, education, income, marital status, alcohol consumption during pregnancy, nonverbal IQ, body mass index (BMI), height, parity, and smoking during pregnancy; child sex; and the IT-HOME score. Potential adjustment variables were selected a priori based on previous studies of OP pesticides and child cognition and on biologically plausible covariate–exposure and covariate–outcome associations observed in our data.[4,9-13,35,36] Finally, to investigate whether the association of DAP concentrations on child IQ differed according to PON1 genotype, the interaction between DAP concentrations and PON1 genotype was formally tested using an a priori criteria for interaction of $p<0.10$. Genetic analyses were carried out in both the full sample and in the Dutch national origin sample.

Sensitivity analyses

First, values below the LOD were substituted with the LOD divided by the square root of 2, instead of using measured values as in the primary analyses. The replacement of values below the LOD with the LOD divided by the square root of 2 is a common substitution method in environmental exposure studies.[37] Second, models were refit with metabolite concentrations expressed as nanomoles per liter with creatinine concentration added as

a separate covariate.[22,38] Third, models truncating the bottom and top 3% of exposure and child nonverbal IQ values were fit to test data robustness. Fourth, the primary models were adjusted for fruit and vegetable intake.[4] Fifth, a multiple informants model was fit as an alternative strategy to model the OP pesticide concentrations collected at three points in time during pregnancy.[39] Sixth, to examine potential selection effects by maternal education, interaction models to assess effect measure modification by maternal education were fit. Seventh, to examine potential sex-specific associations, OP pesticide × child sex interaction models were fit. Eighth, a "complete case" analysis of the data was conducted, utilizing only observations with complete data for all covariates. Ninth, summed models excluding DEDTP metabolites were fit because >80% of concentration values were <LOD. Last, each of the two subtests making up the child nonverbal IQ score (Mosaics and Categories) were modeled as the outcome of interest, in place of the nonverbal IQ score, to assess the specificity of results.

Results

Sample characteristics

Overall, the Generation R Study mothers were on average 30 y of age at enrollment (SD 5 y), and diverse with respect to ethnicity, education, and income (Table 1). Compared with all women in the Generation R Study, the women in the present analysis were more likely to be older, nulliparous, Dutch, highly educated, married, and to occasionally consume alcoholic beverages during pregnancy. Women were also more likely to have a lower BMI and a higher income. Compared with all Generation R Study children who attended the 6-y examination, the IT-HOME scores of our sample were slightly higher. The average DAP concentration in maternal pregnancy urine was higher among those who were older, had a lower BMI, higher income and education, and had partners (Table 1). Child DAP concentrations in urine were weakly associated with those in maternal pregnancy urine ($r<|0.08|$; see also Table S3). The child's DAP concentrations were also slightly positively associated with the HOME scores (not shown). Child nonverbal IQ scores were most strongly related to maternal ethnicity, education, and income and to their IT-HOME score (Table 1).

DAP concentrations

Total DAPs comprised mostly dimethyl alkyl phosphates, and the distribution of concentrations was fairly stable across the three sampling periods (Figure 2). As reported previously, the intraclass correlation coefficient for total DAP concentration across the three phases of pregnancy urine collection in this study was 0.38 (Spaan et al. 2015).

Table 1. Characteristics of all Generation R cohort members and of the participants included in the analysis, and average DAP concentration and non-verbal IQ score by category of characteristics.

Characteristic		Generation R cohort (n=9778) [a]	Included in the analyses (n=708) [a]	Average DAP exposure [b,c]	Child non-verbal IQ score [c,d]
Maternal and infant characteristics at time of enrollment					
Sex of infant at birth					
	Male	50.6 %	51.3 %	378 (263, 511)	103 (16)
	Female	49.4 %	48.7 %	341 (248, 480)	102 (14)
	Missing, n	153	-		
	p-value[e]			0.064	0.289
Age in years					
	< 20	4.2 %	1.7 %	292 (229, 477)	97 (15)
	20-< 25	15.9 %	10.2 %	332 (250, 456)	91 (15)
	25-< 30	26.4 %	26.4 %	324 (245, 476)	103 (15)
	30-< 35	36.9 %	45.8 %	382 (257, 519)	104 (16)
	≥ 35	16.6 %	16.0 %	384 (269, 489)	104 (13)
	Missing, n	-	-		
	p-value[e]			0.076	<0.001
BMI					
	< 18.5	2.1 %	2.4 %	365 (281, 563)	98 (21)
	18.5-< 25	57.9 %	65.4 %	378 (269, 509)	103 (15)
	25-< 30	26.3 %	23.4 %	344 (257, 460)	101 (15)
	≥ 30	13.8 %	8.8 %	262 (196, 427)	99 (15)
	Missing, n	899	3		
	p-value[e]			<0.001	0.047
Height in cm (quartiles)					
	< 161	23.6 %	16.4 %	343 (260, 505)	97 (15)
	161 – < 168	27.4 %	31.0 %	349 (245, 490)	102 (15)
	168 – < 173	24.6 %	25.3 %	343 (245, 489)	103 (15)
	≥ 173	24.4 %	27.3 %	370 (279, 511)	105 (16)
	Missing, n	934	1		
	p-value[e]			0.709	<0.001
Parity (Previous births)					
	0	55.1 %	61.5 %	361 (258, 502)	103 (16)
	1	30.2 %	27.3 %	379 (271, 502)	102 (15)
	≥ 2	14.7 %	11.2 %	295 (216, 429)	100 (14)
	Missing, n	378	4		
	p-value[e]			0.017	0.468
Ethnicity					
	Dutch	50.0 %	57.9 %	370 (253, 487)	106 (15)
	Other Western	11.6 %	12.4 %	365 (283, 514)	101 (15)
	Non-Western	38.4 %	29.7 %	335 (248, 516)	96 (14)
	Missing, n	694	-		
	p-value[e]			0.587	<0.001

Continue

Continued

Characteristic	Generation R cohort (n=9778) [a]	Included in the analyses (n=708) [a]	Average DAP exposure [b,c]	Child non-verbal IQ score [c,d]
Education				
Low (No education finished, Primary education, lower vocational training, intermediate general school or <3 years at general secondary school)	26.5 %	14.8 %	315 (215, 459)	95 (16)
Intermediate (+3 years of secondary education, Intermediate vocational training or first year of higher vocational training)	30.7 %	30.0 %	331 (247, 478)	100 (15)
High (University degree or higher vocational training)	42.8 %	55.2 %	384 (280, 509)	106 (15)
Missing, n	1221	24		
p-value[e]			<0.001	<0.001
Household income in euro's				
<1200 per month	20.7 %	12.6 %	312 (219, 465)	96 (15)
1200–2000 per month	18.5 %	17.0 %	320 (247, 473)	101 (15)
> 2000 per month	60.8 %	70.4 %	381 (278, 501)	105 (15)
Missing, n	3066	90		
p-value[e]			0.007	<0.001
Marital status				
Married/ living with partner	85.5 %	90.3 %	371 (267, 504)	103 (15)
No partner	14.5 %	9.7 %	268 (210, 415)	101 (16)
Missing, n	1213	29		
p-value[e]			<0.001	0.211
IQ score				
≤ 85	16.0 %	18.5 %	321 (219, 477)	96 (14)
>85-≤ 100	43.1 %	44.1 %	353 (263, 507)	102 (15)
>100 -< 115	16.7 %	19.1 %	373 (274, 468)	105 (15)
≥ 115	14.1 %	18.4 %	382 (252, 501)	107 (17)
Missing, n	3427	16		
p-value[e]			0.263	<0.001
Smoking				
No smoking during pregnancy	73.4 %	78.1 %	375 (268, 505)	103 (15)
Until pregnancy recognized	8.6 %	8.5 %	342 (258, 500)	102 (13)
Continued during pregnancy	18.0 %	13.4 %	275 (186, 469)	98 (14)
Missing, n	1534	61		
p-value[e]			0.001	0.008
Alcohol Beverage Consumption				
No alcohol consumption during pregnancy	48.0 %	37.9 %	329 (246, 485)	99 (15)
Until pregnancy recognized	13.2 %	17.3 %	377 (268, 516)	104 (13)
Continued occasionally (less than 1 glass/week)	31.6 %	38.5 %	381 (257, 509)	105 (16)
Continued frequently (1 or more glass/week for at least two trimesters)	7.2 %	6.4 %	349 (295, 461)	106 (14)
Missing, n	1870	37	0.285	<0.001
p-value[e]				

Continue

Continued

Characteristic	Generation R cohort (n=9778) [a]	Included in the analyses (n=708) [a]	Average DAP exposure [b,c]	Child non-verbal IQ score [c,d]
Child at six years				
Total DAP in nmol/g creatinine (quartiles)				
<144	-	24.6 %	341 (248, 483)	100 (16)
144 – < 240	-	24.9 %	332 (244, 457)	103 (14)
240 – < 410	-	25.4 %	377 (266, 503)	102 (14)
≥410	-	25.1 %	380 (268, 519)	104 (17)
Missing, n	-	28		
p-value[e]			0.152	0.140
Household income in euro's				
< 1600 per month	16.5%	15.0%	320 (223, 502)	96 (16)
1600–4000 per month	49.2%	47.0%	344 (252, 475)	101 (16)
> 4000 per month	34.3%	38.0%	390 (287, 529)	107 (14)
Missing, n	3953	40		
p-value[e]			0.001	<0.001
Child's home environments				
IT-HOME score [f] (quartiles)				
<25	36,3%	22.6 %	321 (224, 469)	97 (16)
25–<27	24.9%	26.7 %	362 (258, 505)	101 (15)
27 – >28	18.2%	19.4 %	360 (264, 491)	103 (14)
≥ 28	20,6%	31.3 %	376 (269, 516)	104 (16)
Missing, n	5301	203		
p-value[e]			0.164	0.004

a. Values shown are percentages.
b. Average DAP exposure represents the average exposure during pregnancy (measured at three time points) in nmol/g creatinine. Values shown are medians (25th percentile, 75th percentile).
c. Values presented are based on the study sample (N=708).
d. Values shown are Means (SD).
e. P-value calculated with the use of Kruskal-Wallis test for differences in average DAP concentrations across pregnancy and non-verbal IQ scores by characteristic.
f. Infant-Toddler Home Observation for Measurement of the Environment (IT-HOME) inventory is a 29-item validated measure of the events, objects and social interactions experienced by a child in the family context. IT-HOME was assessed by observation during home visits at average child age of 3.38 months (SD=1.17).

DAP–IQ associations

Overall, the estimated differences in child nonverbal IQ for a log10-unit increase in OP pesticide concentration were inconsistent between adjacent sampling periods (Table 2). The heterogeneity in association across sampling periods was statistically significant for total DAPs and dimethyl alkyl phosphates. For each 10-fold difference in total DAP concentration for the >25 weeks of gestation samples, however, adjusted child nonverbal IQ was 3.9 points lower [95% confidence interval (CI): –7.5, –0.3]. The results for dimethyl alkyl phosphate at >25 weeks of gestation showed inverse associations that were slightly stronger than for total DAPs or diethyl alkyl phosphates, but these estimates

	%<LOD	GM	P05	P25	P50	P75	P95
DEDTP							
< 18 weeks' gestation	80.9%	0.2	0.0	0.0	0.0	0.3	0.9
18 -25 weeks' gestation	83.9%	0.2	0.0	0.0	0.0	0.3	1.1
>25 weeks' gestation	84.3%	0.2	0.0	0.0	0.0	0.3	0.9
DETP							
< 18 weeks' gestation	12.3%	7.9	0.0	2.9	7.3	18.0	67.1
18 -25 weeks' gestation	11.4%	7.5	0.3	2.8	7.0	16.2	51.4
>25 weeks' gestation	11.2%	7.5	0.1	3.1	7.5	16.3	59.6
DEP							
< 18 weeks' gestation	2.7%	32.9	7.2	18.4	32.7	56.5	128.0
18 -25 weeks' gestation	5.2%	31.4	6.8	17.5	31.0	55.1	141.7
>25 weeks' gestation	3.8%	31.4	7.4	16.6	31.9	57.0	138.1
Diethyl alkyl phosphates [a]							
< 18 weeks' gestation		43.7	9.6	25.3	43.4	79.2	178.0
18 -25 weeks' gestation		41.6	9.0	23.3	41.5	76.2	187.0
>25 weeks' gestation		41.6	8.9	21.9	42.1	77.4	182.3
DMDTP							
< 18 weeks' gestation	19.6%	3.5	0.0	1.2	3.0	7.1	30.2
18 -25 weeks' gestation	18.1%	3.8	0.0	1.4	3.3	7.6	29.2
>25 weeks' gestation	17.4%	3.5	0.0	1.3	3.0	6.8	31.4
DMTP							
< 18 weeks' gestation	3.5%	88.7	10.8	49.5	103.2	197.2	490.0
18 -25 weeks' gestation	3.5%	98.1	12.1	63.4	111.1	205.0	458.3
>25 weeks' gestation	2.3%	97.6	14.8	57.0	104.5	193.7	512.4
DMP							
< 18 weeks' gestation	0.1%	132.4	38.9	81.7	131.1	216.9	443.8
18 -25 weeks' gestation	-	138.3	45.0	92.3	138.2	215.5	369.7
>25 weeks' gestation	-	128.1	38.1	82.1	129.8	198.8	403.7
Dimethyl alkyl phosphates [b]							
< 18 weeks' gestation		254.3	72.0	151.3	249.1	419.1	871.8
18 -25 weeks' gestation		268.2	81.1	170.8	269.1	422.6	857.0
>25 weeks' gestation		251.9	70.3	163.0	252.6	403.5	882.2
Total Dialkyl phosphates [c]							
< 18 weeks' gestation		313.8	97.3	191.6	316.0	520.0	1002.9
18 -25 weeks' gestation		322.6	101.2	211.1	316.7	491.9	1001.9
>25 weeks' gestation		307.3	90.2	195.9	310.0	498.5	994.4

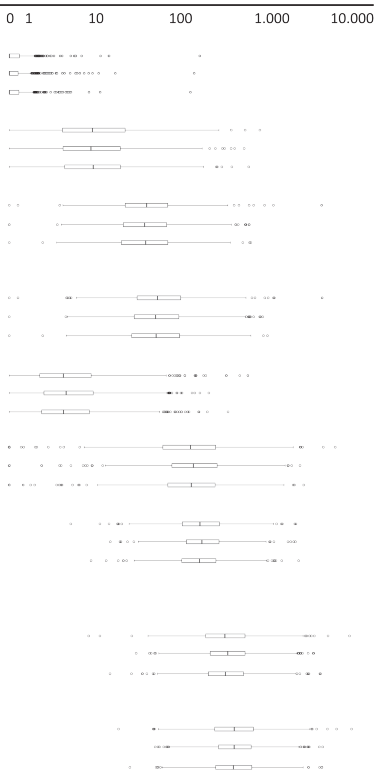

Figure 2. Dialkylphosphate concentrations on a creatinine basis in maternal urine among Generation R participants included in analyses (n=708). Statistics were computed using reported values below the limit of detection. The outer limits of the boxes (left to right) represent the 25th and 75th percentiles; the vertical bars within the boxes represent the 50th percentiles. The whiskers indicate 1.5 times the interquartile range (IQR), and the values more than 1.5 times the IQR are represented as points. Diethyl alkyl phosphates is the sum of DEDTP, DETP, and DEP. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP, and DMP. Total dialkylphosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP, and DMP. Note: DEDTP, diethyldithiophosphate; DEP, diethylphosphate; DETP, diethylthiophosphate; DMDTP, dimethyldithiophosphate; DMP, dimethylphosphate; DMTP, dimethylthiophosphate.

were essentially similar given the width of the 95% CIs for measures at >25 weeks. A representative spline (mean of 10 restricted spline smooths from the MICE models) for total DAP concentration at >25 weeks of gestation is shown in Figure 3 and indicates a slightly steeper and inverse association between exposure and outcome at lower levels of exposure, and the p value for nonlinearity was 0.11. Restricted cubic splines of all 12 exposure–nonverbal IQ associations were largely consistent with results from parametric models (see Figures S1–S3).

Table 2. Difference in cognitive test score at age six (and 95% confidence interval) per log10 nmol/g creatinine increase in maternal urine dialkyl phosphate metabolite concentration, by timing of pregnancy urine sampling and degree of adjustment (n=708).

	Type of adjustment					
	None		Adjusted [b]		Mutually Adjusted [c]	
Dialkyl Phosphate Type	β	(95% CI)	β	(95% CI)	β	(95% CI)
Dialkyl phosphates (total) [a]						
< 18 weeks' gestation	-0.7	-4.1 to 2.8	-1.9	-5.3 to 1.5	-1.8	-5.3 to 1.6
18 – 25 weeks' gestation	3.2	-0.6 to 7.0	0.9	-2.9 to 4.7	2.6	-1.4 to 6.6
> 25 weeks' gestation	-0.8	-4.4 to 2.8	-3.9	-7.5 to -0.3	-4.3	-8.1 to -0.6
Mean of three urines	0.6	-4.3 to 5.5	-2.5	-7.4 to 2.4		-
p homogeneity [d]						0.051
Diethyl alkyl phosphates [e]						
< 18 weeks' gestation	0.1	-2.4 to 2.6	-1.4	-3.8 to 1.1	-1.5	-4.0 to 1.0
18 – 25 weeks' gestation	2.1	-0.4 to 4.7	0.6	-1.9 to 3.1	0.9	-1.7 to 3.5
> 25 weeks' gestation	2.2	-0.3 to 4.7	-0.2	-2.8 to 2.3	-0.3	-2.9 to 2.3
Mean of three urines	4.4	0.6 to 8.2	1.0	-2.8 to 4.9		-
p homogeneity [d]						0.475
Dimethyl alkyl phosphates [f]						
< 18 weeks' gestation	-1.6	-4.9 to 1.7	-2.5	-5.7 to 0.7	-2.3	-5.6 to 0.9
18 – 25 weeks' gestation	2.6	-1.0 to 6.3	0.7	-3.0 to 4.3	2.3	-1.4 to 6.1
> 25 weeks' gestation	-1.6	-5.0 to 1.9	-4.1	-7.5 to -0.7	-4.3	-7.9 to -0.8
Mean of three urines	-0.6	-5.4 to 4.1	-3.0	-7.7 to 1.7		-
p homogeneity [d]						0.030

a. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.
b. Adjusted for age of the mother, sex of child, ethnicity categories (Dutch, other-western and non-western), education (low, intermediate and high), income (low, middle and high), marital status, maternal alcohol consumption (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasionally alcohol consumption during pregnancy and frequently alcohol consumption during pregnancy), maternal non-verbal IQ, BMI categories (<18.5, 18.5-25, 25-30, 30+), height of the mother, parity categories (0, 1, 2+), IT-Home quartiles, and smoking categories (no smoking during pregnancy, smoked until pregnancy was known, smoked during pregnancy).
c. Adjusted model with the inclusion of the three exposures in one model.
d. Multiple-partial F test used to test whether exposure from different time points relates in the same manner to non-verbal IQ scores.
e. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP and DMP.
f. Diethyl alkyl phosphates is the sum of DEDTP, DETP and DEP.

The results among the subset of mother–child pairs with child data on genotype showed that there was no strong statistical support for heterogeneity in the nonverbal child IQ–DAP association by genotype, particularly considering the number of comparisons made (see Tables S4–S11). When the genetic analysis was restricted to Dutch national origin participants, the findings were again unremarkable (see Tables S12–S15).

Sensitivity analyses

As noted above, the sensitivity analyses examined the effects of a) the <LOD substitution method (see Table S16), b) adjustment of creatinine concentration as a separate covariate (see Table S17), c) the removal of extreme exposure and outcome values (see Table S18),

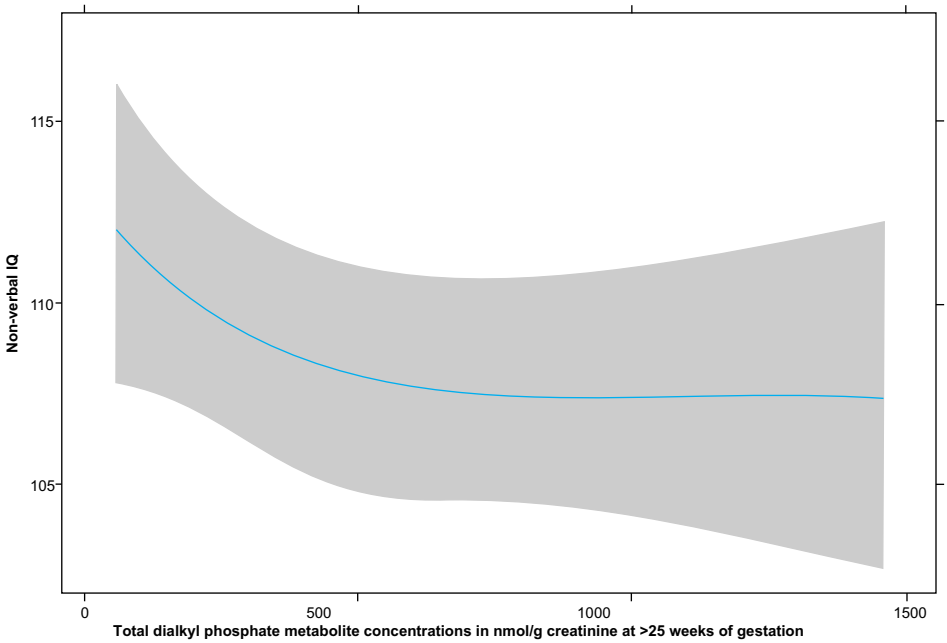

Figure 3. Restricted cubic spline of adjusted child nonverbal IQ scores and (untransformed) total DAP concentration. The solid line represents the estimated mean value of nonverbal IQ scores at each total DAP metabolite concentration, and the shaded area indicates the corresponding 95% confidence band for these estimates. Adjusted for age of the mother, sex of child, ethnicity categories (Dutch, other-Western, and non-Western), education (low, intermediate, and high), income (low, middle, and high), marital status, maternal alcohol consumption (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasional alcohol consumption during pregnancy, and frequent alcohol consumption during pregnancy), maternal nonverbal IQ, BMI categories (<18.5 , 18.5–25, 25–30, >30 kg/m2), height of the mother, parity categories (0, 1, ≥2), IT-Home quartiles, and smoking categories (no smoking during pregnancy, smoked until pregnancy was known, smoked during pregnancy). Note: BMI, body mass index; DAP, dialkylphosphates; IT-HOME, Infant/Toddler Home Observation for Measurement of the Environment inventory.

d) adjustment for prenatal fruit and vegetable consumption (see Table S19), e) analyzing the data using a multiple informants model (see Table S20), f) examining effect measure modification by maternal educational attainment (see Table S21), g) examining effect measure modification by child sex (see Table S22), h) fitting a "complete case" only model (see Table S23–S24), and i) excluding DEDTP (i.e., including only those metabolites with at least 80% of values >LOD) (see Table S25). Finally, Mosaics (see Table S26) and Categories (see Table S27) were modeled as the outcome of interest in place of nonverbal IQ. The sensitivity analyses supported the results shown in Table 2 and the absence of important differences when examining effect measure modification. However, with adjustment for fruit and vegetable intake, the associations tended to be more inverse.

Discussion

In this analysis of data on nonverbal IQ in children in relation to prenatal DAP concentrations in a diverse, urban population in Europe, evidence of an adverse association was weak overall, although there was some suggestion of an inverse relation between nonverbal IQ and late pregnancy urinary DAP concentration. Among the three groups of DAP metabolites analyzed, diethyl metabolites showed the weakest associations with child nonverbal IQ. Where inverse associations were suggested, the adjusted results were generally more strongly inverse than the crude results, consistent with negative confounding.

The results of our study share some consistencies with other published data. For example, in a pooled analysis of data on developmental indices at 2 y of age and DAPs measured in one or two prenatal urine specimens, there was a negative association at lower, as opposed to higher, concentrations of DAPs in three of the four pooled studies (with the fourth study showing linearity throughout the range of exposure), a finding similar to the pattern observed in our data (Figure 3).[13] A larger negative association at lower concentrations was also present in Bouchard's study with IQ measured at 7 y of age 7.[9] In that analysis of 7-y-old children from the CHAMACOS cohort, each 10-fold difference in total DAP concentration—in the second half of pregnancy—was associated with a 3.5-point lower Weschler Intelligence Scale for Children (WISC)-IV Full-Scale IQ score and a 3.1-point lower Verbal Comprehension score.[9] Results from the present study are similar: Each 10-fold difference in total DAP concentration measured at >25 weeks was associated with a 3.9-point lower nonverbal IQ score. Results of our restricted cubic spline models illustrate that associations between DAPs and nonverbal IQ may be stronger at lower levels of exposure. There is evidence from animal and human studies that exposure–disease associations may not be linear.[40] For instance, studies of lead exposure and child IQ have observed nonlinearity in the lead–IQ association across different persons, places, and times.[41-44] For organophosphate pesticides, low-dose developmental toxicity may occur through noncholinergic mechanisms,[8,45] which may have nonlinear dose–effect modes of action.

The timing of the DAP exposure assessment may explain differences across studies. Bouchard et al. (2011) examined child IQ at 7 y of age in relation to urinary DAPs from either the first or second half of pregnancy; the DAP-IQ coefficient was negative for both periods, but more so for the DAPs in the second half of pregnancy.[9] Our results also showed a larger negative association later in pregnancy. Other factors may also explain variation in the results across studies.[13] For example, variation across studies in the degree of confounding by fruit and vegetable intake, or the degree to which urine DAP metabolites concentrations reflect exposure to the active pesticide rather than degradation products, especially in regions where a larger proportion of measured DAP concentrations

is due to agricultural pesticide exposure[46] may explain differences across studies. Other potential reasons for variation may be in the types of pesticides used on food consumed in different countries or in the socioeconomic status (SES) of the studied populations. The authors of two recent studies of organophosphate pesticide metabolites and IQ speculated that a reason for the lack of association in higher SES populations was due to the protective effects of higher SES.[47,48] In the present data, adjusted results were more inverse than the crude results, consistent with the possibility of residual confounding by SES or other lifestyle factors. Additionally, the instruments used to measure IQ differed across studies. The present study utilized a global, nonverbal measure of intelligence. Other studies utilized more complete IQ batteries such as the WISC that may be more sensitive to cognitive deficits because the instrument measures both verbal and nonverbal domains of intellectual function. For instance, in the CHAMACOS cohort, total DAP concentrations were associated with lower scores on the Verbal Comprehension Index, but scores on the Perceptual Reasoning and Processing Speed indices of the WISC-IV were less strongly associated with DAP concentrations, if at all.[35]

In the present study, we did not observe any evidence of effect measure modification by the PON1 gene allele. When we restricted the total sample to only those of Dutch national origin, results also did not show evidence of interaction by PON1 genotype status. In other studies of effect measure modification by genotype, the results on cognitive measures and DAPs have been inconsistent.[11,35] For example, Eskenazi et al. (2014) observed that the association between DAPs and Mental Development Index scores was the strongest in children with PON1-108T allele, but this and other interactions between DAPs and PON1 polymorphisms or enzymes were not statistically significant. On the other hand, Engel et al. (2011) observed a statistically significant interaction (p=0.09), showing a stronger inverse association between log10-DAP exposure and perceptual reasoning in children with the PON192QQ allele. Results based on mother and child genotypes tended to be similar within a given study.[11,35]

The concentration of DAPs in urine only captures short-term exposure, which varies substantially from day to day, depending on diet. Although DAPs were measured at three time points, the average exposure may not have been accurately captured.[17] Furthermore, the proportion of the DAPs measured that reflected exposure to the active pesticide rather than to inactive degradation products was unknown. The rate of degradation of organophosphate pesticides under specific field conditions is hard to predict.[49] of the Generation R population in the present study was higher than for the Generation R cohort as a whole, which may be a reflection of our exclusion criterion of having three urine specimens during pregnancy. Our results therefore may not be generalizable to the Generation R population; however, as noted above, we saw no evidence of effect measure modification by education, suggesting that potential selection bias is unlikely to have

materially affected our results. Among the strengths of our study were the relatively large size, the measurement of maternal DAPs at three time points during pregnancy, and a well-standardized and validated instrument for measuring outcome. Furthermore, as documented elsewhere, the median total DAPs among the Generation R Study mothers was more than 2-fold higher compared with background-exposed pregnant women in the United States living in nonagricultural communities, which suggests a greater range of exposure and statistical power with which to evaluate exposure–disease associations.[50]

Conclusions

Organophosphate pesticide exposure is ubiquitous, and experimental data and evidence from accidental poisoning in humans indicate that it is neurotoxic. The present study, utilizing a well-characterized pregnancy cohort in Rotterdam, Netherlands, examined maternal organophosphate exposure in 708 pregnant women in relation to child nonverbal IQ at 6 y of age. OP pesticide exposure was characterized by six measured dialkylphosphate metabolite concentrations in pregnancy urine specimens at three time points, and child nonverbal IQ was assessed at 6 y of age, after school entry, when IQ tests tend to have greater predictive validity for aspects of learning such as school achievement.[51] Our results suggest that typical, background OP exposures during pregnancy are not consistently associated with lower child IQ at 6 y of age in this population; however, there was some evidence that late pregnancy may be a susceptible period for adverse effects on cognition.

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Supplemental Material

Table S1. Effect allele and single nucleotide polymorphism (SNP) descriptive statistics.

PON1	SNP	Al1	Al2	Freq1	MAF	Quality	R^2
PON1-Q192	rs662	C	T	0.35474	0.35474	0.99993	0.99989
PON1-108	rs705379	G	A	0.56718	0.43282	0.98614	0.95791
PON1-161	rs705381	C	T	0.74361	0.25639	0.99570	0.98359
PON1- L55M	rs854560	A	T	0.67882	0.32118	0.99974	0.99903
PON1-909	rs854572	C	G	0.52449	0.47551	0.99881	0.99633

Note: rs662 was directly genotyped. rs705379, rs705381, rs854560 and rs854572 are based on imputed genotypes.

Table S2. Akaike information criterion (AIC) comparison between untransformed (UTF) and $\log_{10}$ transformed DAP metabolite concentrations at <18 weeks, 18-25 weeks, and >25 weeks.

	UTF <18 weeks	Log_{10} <18 weeks	UTF 18-25 weeks	Log_{10} 18-25 weeks	UTF >25 weeks	Log_{10} >25 weeks	Mean UTF DAP	Mean Log_{10} DAP	Mutually Adjusted UTF DAP	Mutually Adjusted Log_{10} DAP
Imputation set										
1	5825.958	5825.072	5825.344	5826.025	5823.157	5821.785	5825.786	5825.331	5825.071	5823.574
2	5826.598	5825.442	5825.925	5826.666	5823.462	5821.939	5826.434	5826.020	5824.826	5822.834
3	5826.639	5826.015	5826.090	5826.748	5823.600	5822.573	5826.412	5826.123	5825.320	5824.369
4	5827.486	5827.284	5827.329	5828.101	5825.006	5823.170	5827.350	5827.205	5826.170	5824.875
5	5824.194	5823.400	5823.744	5824.393	5822.113	5820.892	5824.175	5823.845	5823.871	5822.498
6	5828.589	5827.536	5828.423	5829.002	5826.163	5824.449	5828.470	5828.001	5827.932	5825.837
7	5825.592	5824.710	5825.191	5825.782	5823.032	5821.161	5825.425	5824.928	5825.028	5822.869
8	5824.786	5823.832	5823.945	5824.831	5821.791	5820.049	5824.652	5824.162	5823.267	5821.500
9	5826.308	5825.438	5825.829	5826.475	5823.164	5821.843	5826.078	5825.800	5824.742	5823.239
10	5826.741	5825.873	5826.505	5826.959	5823.316	5821.546	5826.308	5825.769	5825.646	5823.532
Mean AIC	5826.289	5825.46	5825.833	5826.498	5823.48	5821.941	5826.109	5825.718	5825.187	5823.513

Table S3. Correlation coefficients for maternal and 6-year-old child urine dialkyl phosphate metabolite concentrations (n=708).

Spearman's rho correlations coefficients	< 18 weeks gestation	18 – 25 weeks gestation	> 25 weeks gestation	Child at age 6
Dialkyl phosphates (total)				
< 18 weeks' gestation	1			
18 – 25 weeks' gestation	0.22**	1		
> 25 weeks' gestation	0.17**	0.31**	1	
child at age 6	0.05	0.03	0.04	1
Diethyl alkyl phosphates				
< 18 weeks' gestation	1			
18 – 25 weeks' gestation	0.29**	1		
> 25 weeks' gestation	0.18**	0.28**	1	
child at age 6	0.07	0.01	0.07	1
Dimethyl alkyl phosphates				
< 18 weeks' gestation	1			
18 – 25 weeks' gestation	0.19**	1		
> 25 weeks' gestation	0.15**	0.30**	1	
child at age 6	0.02	0.03	0.02	1
Pearson correlation coefficients (log10)	**< 18 weeks gestation**	**18 – 25 weeks gestation**	**> 25 weeks gestation**	**Child at age 6**
Dialkyl phosphates (total)				
< 18 weeks' gestation	1			
18 – 25 weeks' gestation	0.24**	1		
> 25 weeks' gestation	0.18**	0.32**	1	
child at age 6	-0.01	0.01	-0.01	1
Diethyl alkyl phosphates				
< 18 weeks' gestation	1			
18 – 25 weeks' gestation	0.22**	1		
> 25 weeks' gestation	0.12**	0.23**	1	
child at age 6	0.01	-0.03	0.04	1
Dimethyl alkyl phosphates				
< 18 weeks' gestation	1			
18 – 25 weeks' gestation	0.20**	1		
> 25 weeks' gestation	0.16**	0.31**	1	
child at age 6	-0.02	0.02	-0.01	1

*P<0.05 ** P<0.01.

Table S4. Fully adjusted: Difference in child non-verbal IQ test score at age six years (and 95% confidence interval) per log10 nmol/g creatinine increase in maternal (<18 weeks) urine dialkyl phosphate metabolite concentration by PON1 type (N=474).

		Adjusted models [a]								
		Dialkyl phosphates (total) [b]			Diethyl alkyl phosphates [c]			Dimethyl alkyl phosphates [d]		
	N	β	(95% CI)		β	(95% CI)		β	(95% CI)	
PON1Q192										
QQ (TT)	226	1.3	-4.9 to 7.4		4.2	-1.0 to 9.4		-0.1	-6.0 to 5.8	
QR (CT)	202	-3.2	-9.7 to 3.4		-3.7	-7.7 to 0.3		-3.3	-9.6 to 3.0	
RR (CC) [e]	46	-4.7	-29.6 to 20.3		5.0	-12.7 to 22.6		-6.6	-30.0 to 16.7	
P interaction [f]				0.292			0.042			0.437
PON1L55										
TT	65	-1.0	-15.5 to 13.6		-0.5	-11.1 to 10.2		-0.7	-14.7 to 13.3	
AT	198	-4.7	-12.1 to 2.8		-1.3	-15.1 to 4.4		-5.2	-15.1 to 1.8	
AA	211	0.2	-6.1 to 6.5		-2.1	-6.0 to 1.7		-0.4	-6.4 to 5.7	
P interaction [f]				0.968			0.402			0.982
PON1-108										
TT	90	-3.7	-14.8 to 7.4		1.1	-6.5 to 8.7		-5.5	-16.2 to 5.1	
CT	232	2.0	-4.6 to 8.7		3.3	-2.6 to 9.3		1.3	-5.0 to 7.6	
CC	152	-2.1	-9.9 to 5.7		-3.3	-7.6 to 1.1		-2.3	-9.7 to 5.1	
P interaction [f]				0.722			0.175			0.995
PON1-909										
GG	107	-4.8	-15.1 to 5.5		-0.3	-7.2 to 6.7		-6.0	-15.9 to 3.9	
CG	229	1.8	-4.8 to 8.4		2.3	-3.6 to 8.3		1.3	-4.9 to 7.6	
CC	138	-2.5	-11.2 to 6.1		-2.9	-7.4 to 1.7		-3.2	-11.5 to 5.0	
P interaction [f]				0.677			0.254			0.870
PON1-161										
GG [e]	37	8.4	-13.2 to 30.1		12.3	-6.0 to 30.7		6.5	-13.7 to 26.8	
CG	172	-2.9	-9.7 to 3.9		-0.3	-6.2 to 5.7		-3.1	-9.6 to 3.5	
CC	265	-1.9	-8.1 to 4.2		-2.3	-5.9 to 1.4		-2.8	-8.7 to 3.1	
P interaction [f]				0.599			0.152			0.458

a. Adjusted for child sex, maternal age, ethnicity (Dutch, other-western and non-western), education (low, intermediate, and high), income (low, middle, and high), marital status, maternal alcohol consumption (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasional alcohol consumption during pregnancy, and frequent alcohol consumption during pregnancy), maternal non-verbal IQ, BMI categories (<18.5, 18.5-25, 25-30, and 30+), height of the mother, parity categories (0, 1, and 2+), Home IT quartiles, and smoking categories (no smoking during pregnancy, smoked until pregnancy was known, and smoked during pregnancy).

b. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.

c. Diethyl alkyl phosphates is the sum of DEDTP, DETP and DEP.

d. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP and DMP.

e. BMI included as a continuous variable for PON1Q192 and PON1-161 because of missing levels in BMI for RR and GG.

f. P-value based on interaction where the PON-1 type was treated as an ordinal variable.

Table S5. Fully adjusted: Difference in child non-verbal IQ test score at age six years (and 95% confidence interval) per log10 nmol/g creatinine increase in maternal (18-25 weeks) urine dialkyl phosphate metabolite concentration by PON1 type (N=474).

		Adjusted models [a]								
		Dialkyl phosphates (total) [b]			Diethyl alkyl phosphates [c]			Dimethyl alkyl phosphates [d]		
	N	β	(95% CI)		β	(95% CI)		β	(95% CI)	
PON1Q192										
QQ (TT)	226	2.6	-4.2 to 9.4		1.7	-3.1 to 6.5		2.3	-4.4 to 9.0	
QR (CT)	202	3.7	-4.2 to 11.6		2.5	-1.7 to 6.7		2.7	-4.7 to 10.0	
RR (CC) [e]	46	1.1	-24.3 to 26.6		3.0	-17.6 to 23.6		-1.0	-24.9 to 22.9	
P interaction [f]				0.724			0.910			0.543
PON1L55										
TT	65	8.1	-5.6 to 21.8		8.2	-3.2 to 19.6		7.0	-6.7 to 20.8	
AT	198	1.2	-6.4 to 8.9		-0.9	-7.9 to 5.0		1.6	-7.9 to 9.0	
AA	211	1.5	-6.0 to 9.1		3.0	-0.8 to 6.8		-0.7	-7.8 to 6.5	
P interaction [f]				0.604			0.872			0.381
PON1-108										
TT	90	7.1	-6.4 to 20.7		6.3	-4.3 to 16.9		5.3	-7.4 to 18.1	
CT	232	0.2	-7.0 to 7.4		0.0	-4.0 to 4.0		0.2	-6.8 to 7.1	
CC	152	1.8	-6.7 to 10.3		4.8	-1.2 to 10.8		-0.1	-8.3 to 8.1	
P interaction [f]				0.232			0.951			0.175
PON1-909										
GG	107	3.9	-7.9 to 15.8		4.1	-4.9 to 13.1		2.7	-8.5 to 13.9	
CG	229	1.2	-6.0 to 8.5		0.2	-3.8 to 4.2		1.3	-5.7 to 8.3	
CC	138	2.8	-6.3 to 11.9		5.0	-1.4 to 11.4		0.6	-8.2 to 9.4	
P interaction [f]				0.414			0.808			0.317
PON1-161										
GG [e]	37	6.0	-28.5 to 40.4		-0.1	-16.3 to 16.1		5.0	-29.0 to 38.9	
CG	172	-1.5	-9.6 to 6.6		1.4	-2.7 to 5.5		-1.8	-9.4 to 5.7	
CC	265	4.4	-2.3 to 11.1		3.4	-1.5 to 8.4		3.3	-3.2 to 9.8	
P interaction [f]				0.808			0.920			0.822

a. Adjusted for child sex, maternal age, ethnicity (Dutch, other-western and non-western), education (low, intermediate, and high), income (low, middle, and high), marital status, maternal alcohol consumption (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasional alcohol consumption during pregnancy, and frequent alcohol consumption during pregnancy), maternal non-verbal IQ, BMI categories (<18.5, 18.5-25, 25-30, and 30+), height of the mother, parity categories (0, 1, and 2+), Home IT quartiles, and smoking categories (no smoking during pregnancy, smoked until pregnancy was known, and smoked during pregnancy).

b. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.

c. Diethyl alkyl phosphates is the sum of DEDTP, DETP and DEP.

d. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP and DMP

e. BMI included as a continuous variable for PON1Q192 and PON1-161 because of missing levels in BMI for RR and GG.

f. P-value based on interaction where the PON-1 type was treated as an ordinal variable.

Table S6. Fully adjusted: Difference in child non-verbal IQ test score at age six years (and 95% confidence interval) per log10 nmol/g creatinine increase in maternal (>25 weeks) urine dialkyl phosphate metabolite concentration by PON1 type (N=474).

		Adjusted models [a]								
			Dialkyl phosphates (total) [b]			Diethyl alkyl phosphates [c]			Dimethyl alkyl phosphates [d]		
		N	β	(95% CI)		β	(95% CI)		β	(95% CI)	
PON1Q192											
	QQ (TT)	226	-6.6	-13.4 to 0.1		-0.8	-6.0 to 4.4		-6.7	-13.2 to -0.1	
	QR (CT)	202	3.2	-4.1 to 10.5		2.8	-3.6 to 9.1		2.6	-4.2 to 9.4	
	RR (CC) [e]	46	-7.2	-28.1 to 13.8		-1.3	-16.7 to 14.1		-9.2	-31.5 to 13.1	
	P interaction [f]				0.749			0.927			0.815
PON1L55											
	TT	65	-2.9	-17.0 to 11.3		-3.6	-16.1 to 9.0		-1.9	-14.7 to 11.0	
	AT	198	-5.6	-13.7 to 2.4		1.6	-17.8 to 8.1		-6.0	-17.8 to 1.6	
	AA	211	0.1	-6.2 to 6.5		-1.1	-6.3 to 4.0		0.2	-5.9 to 6.4	
	P interaction [f]				0.383			0.904			0.448
PON1-108											
	TT	90	-5.2	-19.2 to 8.7		5.1	-6.9 to 17.1		-5.9	-18.6 to 6.7	
	CT	232	-0.1	-7.2 to 7.0		-0.2	-5.7 to 5.3		-0.4	-7.1 to 6.4	
	CC	152	-2.6	-9.8 to 4.6		-0.5	-6.6 to 5.5		-2.4	-9.3 to 4.6	
	P interaction [f]				0.757			0.860			0.656
PON1-909											
	GG	107	-5.9	-17.8 to 6.0		-0.9	-10.7 to 9.0		-5.5	-16.5 to 5.4	
	CG	229	0.5	-6.4 to 7.4		1.7	-3.8 to 7.2		-0.1	-6.7 to 6.5	
	CC	138	-2.4	-10.1 to 5.4		-1.2	-7.8 to 5.4		-2.1	-9.5 to 5.4	
	P interaction [f]				0.644			0.883			0.584
PON1-161											
	GG [e]	37	-11.9	-34.6 to 10.7		-16.8	-35.9 to 2.3		-9.3	-32.3 to 13.6	
	CG	172	-2.1	-9.7 to 5.5		4.6	-1.2 to 10.4		-3.8	-11.1 to 3.5	
	CC	265	-2.8	-9.1 to 3.5		-2.4	-7.8 to 2.9		-2.3	-8.2 to 3.6	
	P interaction [f]				0.831			0.676			0.870

a. Adjusted for child sex, maternal age, ethnicity (Dutch, other-western and non-western), education (low, intermediate, and high), income (low, middle, and high), marital status, maternal alcohol consumption (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasional alcohol consumption during pregnancy, and frequent alcohol consumption during pregnancy), maternal non-verbal IQ, BMI categories (<18.5, 18.5-25, 25-30, and 30+), height of the mother, parity categories (0, 1, and 2+), Home IT quartiles, and smoking categories (no smoking during pregnancy, smoked until pregnancy was known, and smoked during pregnancy).

b. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.

c. Diethyl alkyl phosphates is the sum of DEDTP, DETP and DEP.

d. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP and DMP.

e. BMI included as a continuous variable for PON1Q192 and PON1-161 because of missing levels in BMI for RR and GG.

f. P-value based on interaction where the PON-1 type was treated as an ordinal variable.

Table S7. Fully adjusted: Difference in child non-verbal IQ test score at age six years (and 95% confidence interval) per log10 nmol/g creatinine increase in maternal (average pregnancy) urine dialkyl phosphate metabolite concentration by PON1 type (N=474).

		Adjusted models [a]								
			Dialkyl phosphates (total) [b] Averaged across pregnancy			Diethyl alkyl phosphates [c] Averaged across pregnancy			Dimethyl alkyl phosphates [d] Averaged across pregnancy	
		N	β	(95% CI)		β	(95% CI)		β	(95% CI)	
PON1Q192											
	QQ (TT)	226	-2.6	-11.8 to	6.6	2.0	-4.8 to	8.8	-3.6	-12.5 to	5.2
	QR (CT)	202	3.5	-6.2 to	13.1	6.8	-1.5 to	15.2	2.6	-6.5 to	11.7
	RR (CC) [e]	46	-7.7	-35.9 to	20.6	4.3	-15.9 to	24.5	-11.6	-40.5 to	17.4
	P interaction [f]				0.786			0.886			0.800
PON1L55											
	TT	65	1.0	-18.4 to	20.4	1.2	-12.0 to	14.4	1.0	-18.4 to	20.5
	AT	198	-5.0	-15.8 to	5.9	1.8	-20.7 to	10.2	-5.4	-20.7 to	4.8
	AA	211	2.0	-6.5 to	10.5	4.8	-2.3 to	11.9	0.9	-7.2 to	9.0
	P interaction [f]				0.675			0.721			0.724
PON1-108											
	TT	90	-2.5	-20.1 to	15.0	6.3	-6.4 to	19.0	-4.9	-21.5 to	11.8
	CT	232	2.3	-7.3 to	11.8	3.3	-4.3 to	10.8	1.3	-7.9 to	10.5
	CC	152	-1.2	-11.4 to	9.1	3.5	-5.0 to	12.1	-1.6	-11.3 to	8.1
	P interaction [f]				0.631			0.569			0.826
PON1-909											
	GG	107	-5.1	-20.7 to	10.6	2.1	-8.9 to	13.1	-5.8	-20.7 to	9.1
	CG	229	3.1	-6.2 to	12.4	3.4	-4.2 to	11.0	2.1	-6.8 to	11.0
	CC	138	-0.7	-11.9 to	10.5	5.2	-3.9 to	14.3	-1.5	-12.1 to	9.1
	P interaction [f]				0.763			0.888			0.891
PON1-161											
	GG [e]	37	-0.4	-31.7 to	30.8	-4.3	-34.4 to	25.7	0.1	-28.7 to	28.9
	CG	172	-5.1	-15.1 to	4.9	2.7	-5.7 to	11.1	-5.9	-15.3 to	3.5
	CC	265	0.6	-8.2 to	9.3	3.9	-2.8 to	10.5	-0.7	-9.1 to	7.8
	P interaction [f]				0.998			0.650			0.792

a. Adjusted for child sex, maternal age, ethnicity (Dutch, other-western and non-western), education (low, intermediate, and high), income (low, middle, and high), marital status, maternal alcohol consumption (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasional alcohol consumption during pregnancy, and frequent alcohol consumption during pregnancy), maternal non-verbal IQ, BMI categories (<18.5, 18.5-25, 25-30, and 30+), height of the mother, parity categories (0, 1, and 2+), Home IT quartiles, and smoking categories (no smoking during pregnancy, smoked until pregnancy was known, and smoked during pregnancy).

b. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.

c. Diethyl alkyl phosphates is the sum of DEDTP, DETP and DEP.

d. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP and DMP

e. BMI included as a continuous variable for PON1Q192 and PON1-161 because of missing levels in BMI for RR and GG.

f. P-value based on interaction where the PON-1 type was treated as an ordinal variable.

Table S8. Difference in child sex, maternal age, ethnicity, and educational level adjusted child non-verbal IQ test score at age six years (and 95% confidence interval) per log10 nmol/g creatinine increase in maternal (<18 weeks) urine dialkyl phosphate metabolite concentration by PON1 type (N=474).

		Adjusted models [a]								
			Dialkyl phosphates (total) [b] <18 weeks			Diethyl alkyl phosphates [c] <18 weeks			Dimethyl alkyl phosphates [d] <18 weeks	
		N	β	(95% CI)		β	(95% CI)		β	(95% CI)
PON1Q192										
	QQ (TT)	226	1.5	-4.6 to 7.5		4.9	-0.1 to 10.0		0.0	-5.8 to 5.8
	QR (CT)	202	-3.5	-9.7 to 2.6		-3.8	-7.5 to -0.1		-3.5	-9.4 to 2.4
	RR (CC)	46	-0.5	-16.6 to 15.5		0.9	-10.7 to 12.5		-0.6	-15.3 to 14.2
	P interaction [e]				0.361			0.039		0.537
PON1L55										
	TT	65	2.8	-8.7 to 14.4		2.6	-6.7 to 11.9		2.6	-8.4 to 13.6
	AT	198	-3.4	-10.2 to 3.5		-0.3	-13.5 to 5.0		-4.3	-13.5 to 2.3
	AA	211	-1.6	-7.5 to 4.4		-2.3	-5.9 to 1.4		-1.9	-7.5 to 3.8
	P interaction [e]				0.818			0.299		0.866
PON1-108										
	TT	90	-4.6	-14.5 to 5.3		0.6	-6.2 to 7.5		-6.8	-16.4 to 2.8
	CT	232	1.3	-5.0 to 7.7		2.3	-3.2 to 7.8		0.8	-5.3 to 6.9
	CC	152	-3.1	-9.8 to 3.6		-2.9	-6.6 to 0.9		-3.1	-9.5 to 3.3
	P interaction [e]				0.700			0.213		0.992
PON1-909										
	GG	107	-3.9	-13.5 to 5.6		0.0	-6.5 to 6.6		-5.9	-15.1 to 3.4
	CG	229	1.3	-4.9 to 7.5		1.8	-3.6 to 7.3		1.0	-5.0 to 6.9
	CC	138	-3.2	-10.5 to 4.2		-2.4	-6.3 to 1.5		-3.6	-10.6 to 3.4
	P interaction [e]				0.679			0.319		0.891
PON1-161										
	GG	37	4.0	-10.0 to 18.0		11.8	-2.9 to 26.6		3.8	-9.4 to 17.0
	CG	172	-2.2	-8.3 to 3.9		0.0	-5.4 to 5.4		-2.3	-8.2 to 3.5
	CC	265	-2.2	-8.3 to 3.8		-2.2	-5.7 to 1.3		-3.2	-9.0 to 2.6
	P interaction [e]				0.621			0.150		0.465

a. Adjusted for child sex, maternal age, ethnicity (Dutch, other-western, and non-western), and education (low, intermediate, and high).
b. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.
c. Diethyl alkyl phosphates is the sum of DEDTP, DETP and DEP.
d. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP and DMP.
e. P-value based on interaction where the PON-1 type was treated as an ordinal variable.

Table S9. Difference in child sex, maternal age, ethnicity, and educational level adjusted child non-verbal IQ test score at age six years (and 95% confidence interval) per log10 nmol/g creatinine increase in maternal (18-25 weeks) urine dialkyl phosphate metabolite concentration by PON1 type (N=474).

			Adjusted models [a]								
			Dialkyl phosphates (total) [b] 18-25 weeks			Diethyl alkyl phosphates [c] 18-25 weeks			Dimethyl alkyl phosphates [d] 18-25 weeks		
		N	β	(95% CI)		β	(95% CI)		β	(95% CI)	
PON1Q192											
	QQ (TT)	226	1.7	-4.9 to 8.3		1.5	-3.2 to 6.1		1.3	-5.1 to 7.7	
	QR (CT)	202	3.4	-3.9 to 10.7		2.3	-1.6 to 6.3		2.4	-4.5 to 9.3	
	RR (CC)	46	0.4	-16.2 to 16.9		0.5	-11.1 to 12.1		-1.0	-16.6 to 14.7	
	P interaction [e]				0.801			0.887			0.620
PON1L55											
	TT	65	4.5	-8.1 to 17.1		4.4	-5.2 to 14.0		4.1	-8.1 to 16.3	
	AT	198	1.2	-6.0 to 8.4		0.1	-5.9 to 5.6		1.3	-5.9 to 8.2	
	AA	211	0.8	-6.4 to 8.0		2.2	-1.3 to 5.8		-0.9	-7.8 to 5.9	
	P interaction [e]				0.553			0.929			0.348
PON1-108											
	TT	90	7.5	-4.1 to 19.2		6.2	-2.1 to 14.4		5.7	-5.6 to 17.0	
	CT	232	-0.6	-7.3 to 6.2		0.1	-3.7 to 4.0		-0.7	-7.1 to 5.8	
	CC	152	1.8	-5.5 to 9.2		3.8	-1.3 to 8.9		0.4	-6.8 to 7.6	
	P interaction [e]				0.353			0.864			0.279
PON1-909											
	GG	107	4.2	-5.9 to 14.4		4.2	-3.2 to 11.6		2.8	-7.0 to 12.5	
	CG	229	0.1	-6.6 to 6.9		0.2	-3.6 to 4.0		0.1	-6.3 to 6.6	
	CC	138	2.5	-5.3 to 10.4		4.1	-1.3 to 9.6		0.8	-6.9 to 8.5	
	P interaction [e]				0.586			0.741			0.471
PON1-161											
	GG	37	19.3	0.1 to 38.6		4.5	-6.6 to 15.7		17.9	-0.8 to 36.6	
	CG	172	-1.7	-8.9 to 5.6		1.2	-2.5 to 4.9		-1.6	-8.5 to 5.2	
	CC	265	3.2	-3.2 to 9.5		2.8	-1.9 to 7.5		2.2	-4.0 to 8.4	
	P interaction [e]				0.938			0.882			0.977

a. Adjusted for child sex, maternal age, ethnicity (Dutch, other-western, and non-western), and education (low, intermediate, and high).

b. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.

c. Diethyl alkyl phosphates is the sum of DEDTP, DETP and DEP.

d. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP and DMP.

e. P-value based on interaction where the PON-1 type was treated as an ordinal variable.

Table S10. Difference in child sex, maternal age, ethnicity, and educational level adjusted child non-verbal IQ test score at age six years (and 95% confidence interval) per log10 nmol/g creatinine increase in maternal (>25 weeks) urine dialkyl phosphate metabolite concentration by PON1 type (N=474).

		Adjusted models [a]											
		Dialkyl phosphates (total) [b]				Diethyl alkyl phosphates [c]				Dimethyl alkyl phosphates [d]			
		N	β	(95% CI)		β	(95% CI)			β	(95% CI)		
PON1Q192													
	QQ (TT)	226	-7.1	-13.6	to -0.6	-0.5	-5.5	to 4.5		-7.3	-13.6	to -0.9	
	QR (CT)	202	2.8	-3.9	to 9.4	2.7	-3.1	to 8.5		2.3	-3.9	to 8.5	
	RR (CC)	46	-5.4	-20.0	to 9.3	0.4	-9.2	to 9.9		-7.7	-23.2	to 7.8	
	P interaction [e]				0.548				0.849				0.637
PON1L55													
	TT	65	-3.3	-15.3	to 8.8	-3.5	-13.3	to 6.4		-1.7	-13.1	to 9.7	
	AT	198	-5.1	-12.6	to 2.4	2.1	-18.1	to 8.2		-5.7	-18.1	to 1.4	
	AA	211	-0.1	-6.1	to 5.9	-0.4	-5.2	to 4.4		-0.2	-6.0	to 5.7	
	P interaction [e]				0.468				0.947				0.528
PON1-108													
	TT	90	-5.5	-17.5	to 6.6	3.5	-5.9	to 12.8		-6.5	-17.7	to 4.8	
	CT	232	-1.3	-7.8	to 5.2	-0.3	-5.5	to 4.8		-1.4	-7.7	to 4.8	
	CC	152	-1.7	-8.3	to 5.0	1.0	-4.6	to 6.6		-1.7	-8.0	to 4.6	
	P interaction [e]				0.566				0.980				0.481
PON1-909													
	GG	107	-7.1	-18.1	to 3.8	-0.6	-9.0	to 7.7		-7.3	-17.5	to 2.9	
	CG	229	-0.8	-7.2	to 5.6	1.3	-3.8	to 6.4		-1.2	-7.4	to 5.0	
	CC	138	-1.4	-8.5	to 5.7	0.4	-5.7	to 6.4		-1.4	-8.2	to 5.4	
	P interaction [e]				0.495				0.769				0.442
PON1-161													
	GG	37	-0.4	-15.8	to 15.0	-4.0	-17.0	to 8.9		1.0	-14.2	to 16.3	
	CG	172	-2.5	-9.5	to 4.5	3.1	-2.2	to 8.3		-3.5	-10.2	to 3.2	
	CC	265	-3.5	-9.4	to 2.5	-1.2	-6.2	to 3.8		-3.3	-9.0	to 2.3	
	P interaction [e]				0.828				0.805				0.831

a. Adjusted for child sex, maternal age, ethnicity (Dutch, other-western, and non-western), and education (low, intermediate, and high.

b. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.

c. Diethyl alkyl phosphates is the sum of DEDTP, DETP and DEP.

d. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP and DMP.

e. P-value based on interaction where the PON-1 type was treated as an ordinal variable.

Table S11. Difference in child sex, maternal age, ethnicity, and educational level adjusted child non-verbal IQ test score at age six years (and 95% confidence interval) per log10 nmol/g creatinine increase in maternal (average pregnancy) urine dialkyl phosphate metabolite concentration by PON1 type (N=474).

			Adjusted models [a]								
			Dialkyl phosphates (total) [b] Averaged across pregnancy			Diethyl alkyl phosphates [c] Averaged across pregnancy			Dimethyl alkyl phosphates [d] Averaged across pregnancy		
		N	β	(95% CI)		β	(95% CI)		β	(95% CI)	
PON1Q192											
	QQ (TT)	226	-3.4	-12.4 to 5.5		2.6	-3.9 to 9.2		-4.7	-13.2 to 3.8	
	QR (CT)	202	2.7	-6.2 to 11.5		6.0	-1.8 to 13.8		2.0	-6.3 to 10.3	
	RR (CC)	46	-5.0	-24.3 to 14.3		1.2	-12.1 to 14.5		-6.9	-26.6 to 12.7	
	P interaction [e]				0.966			0.988			0.970
PON1L55											
	TT	65	-1.2	-18.3 to 15.9		1.0	-11.0 to 13.1		-0.8	-17.0 to 15.5	
	AT	198	-3.6	-13.6 to 6.4		3.5	-18.2 to 11.3		-4.7	-18.2 to 4.8	
	AA	211	-0.3	-8.3 to 7.6		2.6	-4.0 to 9.3		-1.0	-8.7 to 6.6	
	P interaction [e]				0.805			0.977			0.823
PON1-108											
	TT	90	-3.2	-18.0 to 11.6		5.7	-4.8 to 16.1		-6.2	-20.5 to 8.0	
	CT	232	0.3	-8.7 to 9.3		2.9	-4.2 to 10.0		-0.5	-9.1 to 8.1	
	CC	152	-1.3	-10.2 to 7.5		2.7	-4.8 to 10.2		-1.5	-10.0 to 7.0	
	P interaction [e]				0.852			0.659			0.930
PON1-909											
	GG	107	-4.6	-18.2 to 9.0		3.5	-6.1 to 13.0		-6.5	-19.5 to 6.6	
	CG	229	1.1	-7.6 to 9.8		3.0	-4.1 to 10.1		0.2	-8.1 to 8.5	
	CC	138	-0.8	-10.4 to 8.9		4.2	-3.6 to 12.1		-1.2	-10.4 to 8.0	
	P interaction [e]				0.994			0.984			0.849
PON1-161											
	GG	37	7.5	-11.2 to 26.2		3.1	-14.3 to 20.5		8.7	-9.0 to 26.5	
	CG	172	-4.3	-13.3 to 4.8		1.7	-5.9 to 9.3		-4.6	-13.0 to 3.9	
	CC	265	-0.9	-9.4 to 7.5		4.1	-2.3 to 10.4		-2.4	-10.5 to 5.7	
	P interaction [e]				0.910			0.598			0.668

a. Adjusted for child sex, maternal age, ethnicity (Dutch, other-western, and non-western), and education (low, intermediate, and high).

b. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.

c. Diethyl alkyl phosphates is the sum of DEDTP, DETP and DEP.

d. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP and DMP.

e. P-value based on interaction where the PON-1 type was treated as an ordinal variable.

Table S12. Difference in child sex, maternal age, and educational level adjusted child non-verbal IQ test score at age six years (and 95% confidence interval) per log10 nmol/g creatinine increase in maternal (<18 weeks) urine dialkyl phosphate metabolite concentration in Dutch women, by PON1 type (N=277).

			Adjusted models [a]								
			Dialkyl phosphates (total) [b] <18 weeks			Diethyl alkyl phosphates [c] <18 weeks			Dimethyl alkyl phosphates [d] <18 weeks		
		N	β	(95% CI)		β	(95% CI)		β	(95% CI)	
PON1Q192											
	QQ (TT)	226	0.3	-7.9 to 8.4		5.5	-1.3 to 12.3		-1.6	-9.3 to 6.2	
	QR (CT)	202	-3.3	-12.4 to 5.7		-4.5	-8.7 to -0.2		-2.7	-11.2 to 5.9	
	RR (CC)	46	-8.0	-42.0 to 26.1		0.2	-16.3 to 16.7		-11.2	-40.6 to 18.1	
	P interaction [e]				0.768			0.104			0.921
PON1L55											
	TT	65	-0.4	-16.5 to 15.7		-0.8	-13.5 to 11.9		-0.7	-16.2 to 14.9	
	AT	198	-0.4	-10.0 to 9.2		-0.2	-18.9 to 6.6		-1.4	-18.9 to 7.6	
	AA	211	-5.4	-14.6 to 3.8		-3.2	-7.6 to 1.2		-5.9	-14.6 to 2.8	
	P interaction [e]				0.579			0.502			0.572
PON1-108											
	TT	90	-5.8	-20.3 to 8.7		0.2	-8.2 to 8.5		-8.5	-22.4 to 5.4	
	CT	232	1.6	-7.3 to 10.5		1.3	-6.2 to 8.7		1.3	-7.1 to 9.8	
	CC	152	-5.3	-15.2 to 4.6		-3.9	-8.4 to 0.7		-5.1	-14.4 to 4.3	
	P interaction [e]				0.715			0.280			0.987
PON1-909											
	GG	107	-4.7	-18.9 to 9.5		0.0	-8.1 to 8.1		-7.2	-21.0 to 6.5	
	CG	229	3.6	-4.9 to 12.2		1.5	-6.0 to 9.1		3.5	-4.5 to 11.5	
	CC	138	-8.1	-18.6 to 2.3		-3.9	-8.5 to 0.7		-8.4	-18.3 to 1.6	
	P interaction [e]				0.533			0.295			0.717
PON1-161											
	GG	37	-2.3	-37.8 to 33.3		17.6	-25.4 to 60.6		0.8	-29.1 to 30.8	
	CG	172	-1.3	-10.1 to 7.6		2.0	-5.2 to 9.2		-2.0	-10.4 to 6.4	
	CC	265	-2.8	-11.1 to 5.4		-3.4	-7.5 to 0.6		-3.6	-11.4 to 4.2	
	P interaction [e]				0.772			0.105			0.642

a. Adjusted for child sex, maternal age, and maternal education (low, intermediate, and high).
b. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.
c. Diethyl alkyl phosphates is the sum of DEDTP, DETP and DEP.
d. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP and DMP.
e. P-value based on interaction where the PON-1 type was treated as an ordinal variable.

Table S13. Difference in maternal age, child sex, and educational level adjusted child non-verbal IQ test score at age six years (and 95% confidence interval) per log10 nmol/g creatinine increase in maternal (18-25 weeks) urine dialkyl phosphate metabolite concentration in Dutch women, by PON1 type (N=277).

		Adjusted models [a]											
			Dialkyl phosphates (total) [b] 18-25 weeks				Diethyl alkyl phosphates [c] 18-25 weeks				Dimethyl alkyl phosphates [d] 18-25 weeks			
		N	β	(95% CI)			β	(95% CI)			β	(95% CI)		
PON1Q192														
	QQ (TT)	226	1.5	-7.3	to	10.3	1.2	-4.9	to	7.3	1.2	-7.5	to	9.9
	QR (CT)	202	4.0	-6.0	to	14.0	2.1	-2.6	to	6.8	3.1	-6.2	to	12.5
	RR (CC)	46	1.0	-40.0	to	42.0	-0.2	-22.6	to	22.3	0.2	-42.8	to	43.3
	P interaction [e]					0.723				0.642				0.820
PON1L55														
	TT	65	13.2	-4.4	to	30.9	8.3	-4.3	to	20.8	12.3	-6.1	to	30.7
	AT	198	1.3	-8.4	to	10.9	-0.2	-10.3	to	7.2	1.8	-10.3	to	11.2
	AA	211	0.8	-9.8	to	11.3	2.1	-2.3	to	6.5	-0.9	-10.8	to	9.0
	P interaction [e]					0.417				0.922				0.301
PON1-108														
	TT	90	1.9	-12.5	to	16.4	3.1	-7.2	to	13.5	1.1	-13.3	to	15.5
	CT	232	2.9	-6.7	to	12.4	1.1	-3.2	to	5.3	2.3	-6.9	to	11.5
	CC	152	-1.3	-12.9	to	10.3	3.5	-4.9	to	11.9	-2.2	-13.3	to	8.9
	P interaction [e]					0.422				0.999				0.365
PON1-909														
	GG	107	3.4	-10.3	to	17.2	4.3	-5.4	to	14.0	2.4	-11.0	to	15.7
	CG	229	3.5	-5.7	to	12.6	0.9	-3.3	to	5.0	3.7	-5.2	to	12.6
	CC	138	-1.7	-14.6	to	11.1	3.5	-5.8	to	12.9	-3.1	-15.2	to	9.0
	P interaction [e]					0.403				0.816				0.349
PON1-161														
	GG	37	13.9	-25.1	to	53.0	1.4	-25.9	to	28.7	14.0	-21.5	to	49.5
	CG	172	-6.4	-15.9	to	3.1	0.6	-3.4	to	4.7	-6.2	-15.3	to	3.0
	CC	265	7.6	-1.2	to	16.5	4.8	-1.8	to	11.4	6.8	-1.9	to	15.4
	P interaction [e]					0.118				0.287				0.134

a. Adjusted for child sex, maternal age, and education (low, intermediate, and high).

b. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.

c. Diethyl alkyl phosphates is the sum of DEDTP, DETP and DEP.

d. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP and DMP.

e. P-value based on interaction where the PON-1 type was treated as an ordinal variable.

Table S14. Difference in child sex, maternal age, and educational level adjusted child non-verbal IQ test score at age six years (and 95% confidence interval) per log10 nmol/g creatinine increase in maternal (>25 weeks) urine dialkyl phosphate metabolite concentration in Dutch women, by PON1 type (N=277).

		Adjusted models [a]								
		Dialkyl phosphates (total) [b]			Diethyl alkyl phosphates [c]			Dimethyl alkyl phosphates [d]		
	N	β	(95% CI)		β	(95% CI)		β	(95% CI)	
PON1Q192										
QQ (TT)	226	-5.9	-15.3 to 3.6		-0.9	-8.1 to 6.2		-6.6	-15.6 to 2.5	
QR (CT)	202	1.7	-7.2 to 10.6		3.4	-4.4 to 11.2		1.0	-7.4 to 9.3	
RR (CC)	46	-14.4	-44.9 to 16.1		-3.2	-17.5 to 11.0		-23.1	-66.5 to 20.3	
P interaction [e]				0.541			0.592			0.626
PON1L55										
TT	65	-6.4	-23.5 to 10.6		8.7	-7.4 to 24.7		-7.7	-22.8 to 7.4	
AT	198	-3.8	-14.9 to 7.3		1.7	-27.5 to 10.1		-4.7	-27.5 to 5.8	
AA	211	1.1	-7.4 to 9.7		-0.6	-7.5 to 6.2		1.0	-7.2 to 9.2	
P interaction [e]				0.500			0.302			0.355
PON1-108										
TT	90	-11.2	-27.9 to 5.5		8.2	-5.7 to 22.1		-13.3	-28.5 to 2.0	
CT	232	4.2	-5.2 to 13.6		0.5	-6.3 to 7.3		3.1	-5.9 to 12.2	
CC	152	-2.2	-12.2 to 7.9		-1.2	-10.0 to 7.5		-1.4	-10.8 to 8.1	
P interaction [e]				0.782			0.238			0.480
PON1-909										
GG	107	-12.1	-27.5 to 3.4		3.7	-8.7 to 16.1		-13.4	-27.6 to 0.8	
CG	229	5.3	-3.7 to 14.3		2.2	-4.5 to 8.9		4.1	-4.6 to 12.7	
CC	138	-3.6	-14.4 to 7.1		-4.3	-13.9 to 5.4		-2.5	-12.5 to 7.6	
P interaction [e]				0.760			0.247			0.479
PON1-161										
GG	37	-5.1	-43.1 to 33.0		-10.8	-39.0 to 17.5		-2.8	-41.7 to 36.1	
CG	172	-5.1	-13.9 to 3.6		-0.7	-7.7 to 6.3		-5.7	-14.1 to 2.7	
CC	265	2.2	-6.8 to 11.1		3.4	-3.7 to 10.5		1.0	-7.4 to 9.4	
P interaction [e]				0.267			0.145			0.342

a. Adjusted for child sex, maternal age, and education (low, intermediate, and high).
b. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.
c. Diethyl alkyl phosphates is the sum of DEDTP, DETP and DEP.
d. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP and DMP.
e. P-value based on interaction where the PON-1 type was treated as an ordinal variable.

Table S15. Difference in child sex, maternal age, and educational level adjusted child non-verbal IQ test score at age six years (and 95% confidence interval) per log10 nmol/g creatinine increase in maternal (average pregnancy) urine dialkyl phosphate metabolite concentration in Dutch women, by PON1 type (N=277).

			Adjusted models [a]								
			Dialkyl phosphates (total) [b] Averaged across pregnancy			Diethyl alkyl phosphates [c] Averaged across pregnancy			Dimethyl alkyl phosphates [d] Averaged across pregnancy		
		N	β	(95% CI)		β	(95% CI)		β	(95% CI)	
PON1Q192											
	QQ (TT)	226	-3.4	-16.7 to 9.8		3.6	-5.6 to 12.8		-5.7	-18.3 to 6.9	
	QR (CT)	202	2.7	-9.8 to 15.1		7.2	-3.1 to 17.4		1.9	-9.7 to 13.4	
	RR (CC)	46	-15.2	-57.1 to 26.8		-1.1	-22.9 to 20.8		-22.7	-72.4 to 27.0	
	P interaction [e]				0.716			0.636			0.708
PON1L55											
	TT	65	-4.2	-32.9 to 24.6		7.8	-10.8 to 26.4		-8.4	-36.7 to 20.0	
	AT	198	-0.4	-15.1 to 14.4		4.0	-29.4 to 14.6		-1.5	-29.4 to 12.2	
	AA	211	-0.5	-12.7 to 11.7		5.8	-4.0 to 15.5		-1.9	-13.4 to 9.6	
	P interaction [e]				0.961			0.988			0.900
PON1-108											
	TT	90	-9.7	-30.9 to 11.5		7.2	-7.0 to 21.5		-13.4	-33.2 to 6.4	
	CT	232	7.3	-6.6 to 21.2		5.1	-4.6 to 14.8		5.8	-7.6 to 19.1	
	CC	152	-3.8	-17.4 to 9.8		2.2	-9.6 to 14.0		-3.6	-16.2 to 9.0	
	P interaction [e]				0.807			0.409			0.912
PON1-909											
	GG	107	-9.1	-29.4 to 11.2		7.5	-5.8 to 20.9		-12.1	-31.1 to 7.0	
	CG	229	10.6	-2.5 to 23.6		4.3	-5.4 to 13.9		9.3	-3.2 to 21.8	
	CC	138	-7.0	-21.6 to 7.7		2.4	-10.1 to 15.0		-6.7	-20.3 to 6.8	
	P interaction [e]				0.706			0.439			0.928
PON1-161											
	GG	37	-1.0	-46.1 to 44.0		-5.1	-52.8 to 42.6		2.9	-35.5 to 41.2	
	CG	172	-8.6	-21.5 to 4.4		0.1	-9.7 to 9.8		-9.0	-21.1 to 3.2	
	CC	265	6.1	-6.4 to 18.7		8.8	-0.3 to 17.8		3.8	-8.1 to 15.7	
	P interaction [e]				0.233			0.141			0.368

a. Adjusted for child sex, maternal age, education (low, intermediate and high).

b. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.

c. Diethyl alkyl phosphates is the sum of DEDTP, DETP and DEP.

d. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP and DMP.

e. P-value based on interaction where the PON-1 type was treated as an ordinal variable.

Table S16. Concentrations <LOD substituted with LOD/√2: Difference in child non-verbal IQ test score at age six years (and 95% confidence interval) per log10 nmol/g creatinine increase in maternal urine dialkyl phosphate metabolite concentration [a], by timing of pregnancy urine sampling and degree of adjustment (N=708).

	Type of adjustment								
	None			Adjusted [b]			Mutually adjusted [c]		
Dialkyl Phosphate Type	β	(95% CI)		β	(95% CI)		β	(95% CI)	
Dialkyl phosphates (total) [d]									
< 18 weeks' gestation	-0.6	-4.1	to 2.9	-1.9	-5.3	to 1.5	-1.8	-5.3	to 1.7
18 – 25 weeks' gestation	3.2	-0.6	to 7.0	0.9	-2.9	to 4.7	2.6	-1.4	to 6.7
> 25 weeks' gestation	-0.8	-4.5	to 2.8	-3.9	-7.5	to -0.3	-4.3	-8.1	to -0.6
Mean of three urines	0.6	-4.4	to 5.5	-2.5	-7.4	to 2.4			
Diethyl alkyl phosphates [e]									
< 18 weeks' gestation	2.5	-0.4	to 5.5	0.5	-2.4	to 3.4	0.5	-2.5	to 3.5
18 – 25 weeks' gestation	2.7	-0.2	to 5.7	0.4	-2.5	to 3.3	0.4	-2.7	to 3.5
> 25 weeks' gestation	2.6	-0.4	to 5.5	-0.3	-3.3	to 2.6	-0.5	-3.5	to 2.5
Mean of three urines	4.3	0.5	to 8.2	1.0	-2.9	to 4.8			
Dimethyl alkyl phosphates [f]									
< 18 weeks' gestation	-1.6	-4.9	to 1.8	-2.4	-5.7	to 0.8	-2.3	-5.7	to 1.0
18 – 25 weeks' gestation	2.7	-1.0	to 6.4	0.7	-2.9	to 4.3	2.4	-1.4	to 6.2
> 25 weeks' gestation	-1.5	-5.0	to 1.9	-4.1	-7.5	to -0.6	-4.3	-7.9	to -0.8
Mean of three urines	-0.6	-5.4	to 4.1	-3.0	-7.7	to 1.7			

a. Values below the limit of detection (LOD) substituted by the LOD/√2.
b. Adjusted for child sex, maternal age, ethnicity (Dutch, other-western, and non-western), education (low, intermediate, and high), income (low, middle, and high), marital status, maternal alcohol consumption (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasional alcohol consumption during pregnancy, and frequent alcohol consumption during pregnancy), maternal non-verbal IQ, BMI categories (<18.5, 18.5-25, 25-30, and 30+), height of the mother, parity categories (0, 1, and 2+), IT-Home quartiles, and smoking categories (no smoking during pregnancy, smoked until pregnancy was known, and smoked during pregnancy).
c. Adjusted model with the inclusion of the three exposures in one model.
d. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.
e. Diethyl alkyl phosphates is the sum of DEDTP, DETP and DEP.
f. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP and DMP.

Table S17. Creatinine adjustment as separate covariate: Difference in child non-verbal IQ test score at age six years (and 95% confidence interval) per log10 nmol/g creatinine increase in maternal urine dialkyl phosphate metabolite concentration, by timing of pregnancy urine sampling and degree of adjustment (N=708).

	Type of adjustment											
	None [a]				Adjusted [b]				Mutually adjusted [c]			
Dialkyl Phosphate Type	β	(95% CI)			β	(95% CI)			β	(95% CI)		
Dialkyl phosphates (total) [d]												
< 18 weeks' gestation	-1.8	-5.1	to	1.5	-2.4	-5.5	to	0.8	-2.5	-5.7	to	0.7
18 – 25 weeks' gestation	3.2	-0.3	to	6.6	0.9	-2.5	to	4.3	2.7	-0.8	to	6.3
> 25 weeks' gestation	-1.8	-5.3	to	1.6	-3.9	-7.3	to	-0.5	-4.5	-8.0	to	-1.0
Mean of three urines	-0.7	-5.5	to	4.1	-2.6	-7.3	to	2.1				
Diethyl alkyl phosphates [e]												
< 18 weeks' gestation	-0.4	-2.8	to	2.1	-1.6	-4.0	to	0.9	-1.6	-4.1	to	0.8
18 – 25 weeks' gestation	2.3	-0.1	to	4.8	0.6	-1.9	to	3.0	1.0	-1.5	to	3.5
> 25 weeks' gestation	1.6	-0.9	to	4.1	-0.5	-3.0	to	2.0	-0.6	-3.1	to	2.0
Mean of three urines	3.0	-0.6	to	6.7	0.5	-3.2	to	4.1				
Dimethyl alkyl phosphates [f]												
< 18 weeks' gestation	-2.5	-5.7	to	0.6	-2.8	-5.8	to	0.2	-2.9	-6.0	to	0.1
18 – 25 weeks' gestation	2.7	-0.6	to	6.1	0.7	-2.6	to	4.0	2.6	-0.8	to	6.0
> 25 weeks' gestation	-2.4	-5.7	to	0.9	-4.0	-7.3	to	-0.8	-4.6	-7.9	to	-1.3
Mean of three urines	-1.9	-6.6	to	2.7	-3.3	-7.8	to	1.3				

a. "None" adjusted model is adjusted for creatinine concentrations.
b. Adjusted for creatinine concentrations, child sex, maternal age, ethnicity (Dutch, other-western, and non-western), education (low, intermediate, and high), income (low, middle, and high), marital status, maternal alcohol consumption (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasional alcohol consumption during pregnancy, and frequent alcohol consumption during pregnancy), maternal non-verbal IQ, BMI categories (<18.5, 18.5-25, 25-30, and 30+), height of the mother, parity categories (0, 1, and 2+), IT-Home quartiles, and smoking categories (no smoking during pregnancy, smoked until pregnancy was known, and smoked during pregnancy).
c. Adjusted model with the inclusion of the three exposures in one model.
d. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.
e. Diethyl alkyl phosphates is the sum of DEDTP, DETP and DEP.
f. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP and DMP.

Table S18. Removal of extreme values: Difference in child non-verbal IQ test score at age six years (and 95% confidence interval) per log10 nmol/g creatinine increase in maternal urine dialkyl phosphate metabolite concentration, by timing of pregnancy urine sampling and degree of adjustment.

	Type of adjustment											
	None				Adjusted [a]				Mutually adjusted [b]			
Dialkyl Phosphate Type	β	(95% CI)			β	(95% CI)			β	(95% CI)		
Dialkyl phosphates (total) [c]												
< 18 weeks' gestation (n=639)	1.2	-2.1	to	4.4	-0.5	-3.7	to	2.7	-0.5	-3.6	to	2.7
18 – 25 weeks' gestation (n=638)	2.4	-1.2	to	5.9	0.6	-2.9	to	4.2	2.9	-0.7	to	6.4
> 25 weeks' gestation (n=640)	0.3	-3.0	to	3.7	-2.8	-6.2	to	0.6	-2.8	-6.2	to	0.6
Mean of three urines (n=639)	3.5	-1.2	to	8.1	0.4	-4.4	to	5.1				
Diethyl alkyl phosphates [d]												
< 18 weeks' gestation (n=638)	2.1	-0.7	to	4.9	-0.2	-3.0	to	2.6	0.7	-2.0	to	3.4
18 – 25 weeks' gestation (n=639)	1.5	-0.7	to	3.8	0.2	-2.0	to	2.5	0.0	-2.3	to	2.3
> 25 weeks' gestation (n=640)	1.8	-0.4	to	4.1	-0.3	-2.6	to	2.0	-1.2	-3.5	to	1.0
Mean of three urines (n=638)	2.1	-1.4	to	5.7	-1.0	-4.7	to	2.6				
Dimethyl alkyl phosphates [e]												
< 18 weeks' gestation (n=640)	0.5	-2.6	to	3.6	-0.8	-3.8	to	2.3	-0.9	-3.9	to	2.1
18 – 25 weeks' gestation (n=638)	1.8	-1.6	to	5.2	0.5	-2.9	to	3.9	2.5	-0.9	to	5.9
> 25 weeks' gestation (n=640)	-0.2	-3.4	to	3.0	-2.7	-6.0	to	0.5	-2.9	-6.2	to	0.4
Mean of three urines (n=638)	2.3	-2.2	to	6.8	0.0	-4.5	to	4.6				

a. Adjusted for child sex, maternal age, ethnicity (Dutch, other-western, and non-western), education (low, intermediate, and high), income (low, middle, and high), marital status, maternal alcohol consumption (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasional alcohol consumption during pregnancy, and frequent alcohol consumption during pregnancy), maternal non-verbal IQ, BMI categories (<18.5, 18.5-25, 25-30, and 30+), height of the mother, parity categories (0, 1, and 2+), IT-Home quartiles, and smoking categories (no smoking during pregnancy, smoked until pregnancy was known, and smoked during pregnancy).

b. Adjusted model with the inclusion of the three exposures in one model.

c. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.

d. Diethyl alkyl phosphates is the sum of DEDTP, DETP and DEP.

e. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP and DMP.

Table S19. Adjustment for prenatal fruit and vegetable consumption: Difference in child non-verbal IQ test score at age six years (and 95% confidence interval) per log10 nmol/g creatinine increase in maternal urine dialkyl phosphate metabolite concentration, by timing of pregnancy urine sampling and degree of adjustment (N=708).

	Type of adjustment								
	None			Adjusted [a]			Mutually adjusted [b]		
Dialkyl Phosphate Type	β	(95% CI)		β	(95% CI)		β	(95% CI)	
Dialkyl phosphates (total) [c]									
< 18 weeks' gestation	-0.7	-4.1	to 2.8	-2.5	-5.9	to 1.0	-2.4	-5.9	to 1.2
18 – 25 weeks' gestation	3.2	-0.6	to 7.0	0.8	-3.1	to 4.6	2.5	-1.5	to 6.6
> 25 weeks' gestation	-0.8	-4.4	to 2.8	-4.0	-7.6	to -0.3	-4.3	-8.1	to -0.5
Mean of three urines	0.6	-4.3	to 5.5	-2.9	-7.9	to 2.0			
Diethyl alkyl phosphates [d]									
< 18 weeks' gestation	0.1	-2.4	to 2.6	-1.6	-4.1	to 0.9	-1.7	-4.3	to 0.8
18 – 25 weeks' gestation	2.1	-0.4	to 4.7	0.5	-2.0	to 3.0	0.9	-1.7	to 3.5
> 25 weeks' gestation	2.2	-0.3	to 4.7	-0.1	-2.7	to 2.4	-0.1	-2.8	to 2.5
Mean of three urines	4.4	0.6	to 8.2	0.9	-3.0	to 4.8			
Dimethyl alkyl phosphates [c]									
< 18 weeks' gestation	-1.6	-4.9	to 1.7	-3.1	-6.3	to 0.2	-2.9	-6.2	to 0.4
18 – 25 weeks' gestation	2.6	-1.0	to 6.3	0.5	-3.2	to 4.1	2.2	-1.6	to 6.0
> 25 weeks' gestation	-1.6	-5.0	to 1.9	-4.2	-7.6	to -0.7	-4.3	-7.9	to -0.8
Mean of three urines	-0.6	-5.4	to 4.1	-3.5	-8.3	to 1.3			

a. Adjusted for child sex, maternal age, ethnicity (Dutch, other-western, and non-western), education (low, intermediate, and high), income (low, middle, and high), marital status, maternal alcohol consumption (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasional alcohol consumption during pregnancy, and frequent alcohol consumption during pregnancy), maternal non-verbal IQ, BMI categories (<18.5, 18.5-25, 25-30, and 30+), height of the mother, parity categories (0, 1, and 2+), IT-Home quartiles, smoking categories (no smoking during pregnancy, smoked until pregnancy was known, and smoked during pregnancy), fruit consumption in grams, vegetables consumption in grams, and energy intake in kcal.

b. Adjusted model with the inclusion of the three exposures in one model.

c. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.

d. Diethyl alkyl phosphates is the sum of DEDTP, DETP and DEP.

e. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP and DMP.

Table S20. Multiple informants model: Difference in child non-verbal IQ test score at age six years (and 95% confidence interval) per log10 nmol/g creatinine increase in maternal urine dialkyl phosphate metabolite concentration, by timing of pregnancy urine sampling (N=708).

	Estimates based on the multiple informants approach [a]		
Dialkyl Phosphate Type	β	(95% CI)	
Dialkyl phosphates (total) [b]			
< 18 weeks' gestation	-2.0	-5.3 to 1.4	
18 – 25 weeks' gestation	0.8	-2.9 to 4.5	
> 25 weeks' gestation	-4.0	-7.5 to -0.4	
p homogeneity [c]			0.049
Diethyl alkyl phosphates [d]			
< 18 weeks' gestation	-1.4	-3.9 to 1.0	
18 – 25 weeks' gestation	0.6	-1.9 to 3.0	
> 25 weeks' gestation	-0.2	-2.7 to 2.3	
p homogeneity [c]			0.722
Dimethyl alkyl phosphates [e]			
< 18 weeks' gestation	-2.5	-5.7 to 0.6	
18 – 25 weeks' gestation	0.6	-3.0 to 4.1	
> 25 weeks' gestation	-4.2	-7.5 to -0.8	
p homogeneity [c]			0.020

a. Adjusted for child sex, maternal age, ethnicity (Dutch, other-western, and non-western), education (low, intermediate, and high), income (low, middle, and high), marital status, maternal alcohol consumption (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasional alcohol consumption during pregnancy, and frequent alcohol consumption during pregnancy), maternal non-verbal IQ, BMI categories (<18.5, 18.5-25, 25-30, and 30+), height of the mother, parity categories (0, 1, and 2+), IT-Home quartiles, and smoking categories (no smoking during pregnancy, smoked until pregnancy was known, and smoked during pregnancy).

b. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.

c. Tests whether the exposure from different time points relates in the same manner to non-verbal IQ scores.

d. Diethyl alkyl phosphates is the sum of DEDTP, DETP and DEP.

e. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP and DMP.

Table S21. Effect measure modification by maternal education: Difference in child non-verbal IQ test score at age six years (and 95% confidence interval) per log10 nmol/g creatinine increase in maternal urine dialkyl phosphate metabolite concentration, by timing of pregnancy urine sampling, degree of adjustment, and maternal education level (N=708).

	Educational level											
	High				Middle				Low			
Total Dialkyl phosphates[a]	B	95%CI			B	95%CI			B	95%CI		P interaction
Type of adjustment												
None												
< 18 weeks' gestation	0.0	-5.0	to	5.0	-6.1	-12.1	to	-0.2	1.0	-6.8	to	8.9	0.836
18 – 25 weeks' gestation	2.0	-3.4	to	7.3	-1.4	-8.1	to	5.2	2.3	-6.5	to	11.0	0.796
> 25 weeks' gestation	-6.1	-11.2	to	-1.0	-1.3	-7.4	to	4.8	-1.8	-10.2	to	6.6	0.341
Mean of three urines	-2.7	-9.8	to	4.5	-4.8	-13.2	to	3.5	1.2	-10.0	to	12.3	0.745
Adjusted [b]												
< 18 weeks' gestation	-1.2	-6.3	to	3.9	-7.3	-13.7	to	-0.9	2.7	-6.7	to	12.1	0.675
18 – 25 weeks' gestation	1.3	-4.1	to	6.7	-1.1	-8.4	to	6.1	1.8	-9.3	to	12.8	0.944
> 25 weeks' gestation	-5.8	-11.0	to	-0.7	-3.9	-10.6	to	2.8	-0.2	-10.6	to	10.2	0.241
Mean of three urines	-3.5	-10.8	to	3.9	-7.3	-16.5	to	1.9	3.4	-10.4	to	17.1	0.398
Mutually adjusted [c]												
< 18 weeks' gestation	-0.9	-6.1	to	4.3	-7.2	-13.8	to	-0.7	2.5	-7.6	to	12.7	0.700
18 – 25 weeks' gestation	3.3	-2.3	to	8.9	1.7	-6.0	to	9.3	0.9	-11.3	to	13.2	0.986
> 25 weeks' gestation	-6.5	-11.9	to	-1.2	-3.5	-10.5	to	3.4	-0.8	-11.8	to	10.1	0.257

a. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.

b. Adjusted for child sex, maternal age, ethnicity (Dutch, other-western, and non-western), income (low, middle, and high), marital status, maternal alcohol consumption (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasional alcohol consumption during pregnancy, and frequent alcohol consumption during pregnancy), maternal non-verbal IQ, BMI categories (<18.5, 18.5-25, 25-30, and 30+), height of the mother, parity categories (0, 1, and 2+), Home IT quartiles, and smoking categories (no smoking during pregnancy, smoked until pregnancy was known, and smoked during pregnancy).

c. Adjusted model with the inclusion of the three exposures in one model.

Table S22. Effect measure modification by child sex: Difference in child non-verbal IQ test score at age six years (and 95% confidence interval) per log10 nmol/g creatinine increase in maternal urine dialkyl phosphate metabolite concentration, by timing of pregnancy urine sampling, degree of adjustment, and child sex (N=708).

	Child sex									
	Boys					Girls				
Dialkyl phosphates (total) [a]	B	95%CI				B	95%CI			P interaction
Type of adjustment										
None										
< 18 weeks' gestation	-0.4	-5.5	to	4.7		-1.1	-5.9	to	3.6	0.844
18 – 25 weeks' gestation	1.9	-3.6	to	7.3		4.5	-0.8	to	9.8	0.499
> 25 weeks' gestation	0.0	-5.4	to	5.4		-1.7	-6.5	to	3.1	0.651
Mean of three urines	0.5	-6.6	to	7.6		0.3	-6.4	to	7.1	0.976
Adjusted [b]										
< 18 weeks' gestation	-2.0	-7.2	to	3.2		-1.8	-6.3	to	2.8	0.840
18 – 25 weeks' gestation	-0.3	-5.9	to	5.2		2.1	-3.3	to	7.4	0.619
> 25 weeks' gestation	-3.0	-8.5	to	2.4		-5.3	-10.2	to	-0.3	0.626
Mean of three urines	-2.6	-9.9	to	4.7		-2.7	-9.5	to	4.2	0.979
Mutually adjusted [c]										
< 18 weeks' gestation	-1.8	-7.3	to	3.7		-1.6	-6.2	to	3.0	0.751
18 – 25 weeks' gestation	0.9	-5.0	to	6.8		4.6	-1.0	to	10.2	0.550
> 25 weeks' gestation	-3.0	-8.6	to	2.7		-6.5	-11.7	to	-1.3	0.566

a. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.
b. Adjusted for maternal age, ethnicity categories (Dutch, other-western, and non-western), education (low, intermediate, and high), income (low, middle, and high), marital status, maternal alcohol consumption (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasional alcohol consumption during pregnancy, and frequent alcohol consumption during pregnancy), maternal non-verbal IQ, BMI categories (<18.5, 18.5-25, 25-30, and 30+), height of the mother, parity categories (0, 1, and 2+), Home IT quartiles, and smoking categories (no smoking during pregnancy, smoked until pregnancy was known, and smoked during pregnancy).
c. Adjusted model with the inclusion of the three exposures in one model.

Table S23. Complete-case only model (with the exclusion of IT-Home score): Difference in child non-verbal IQ test score at age six years (and 95% confidence interval) per log10 nmol/g creatinine increase in maternal urine dialkyl phosphate metabolite concentration, by timing of pregnancy urine sampling, and degree of adjustment, among mother-child pairs with complete covariate data.

	Type of adjustment											
	None (n=708)				Adjusted (n=541) [a]				Mutually adjusted (n=541) [b]			
Dialkyl Phosphate Type	β	(95% CI)			β	(95% CI)			β	(95% CI)		
Dialkyl phosphates (total) [c]												
< 18 weeks' gestation	-0.7	-4.2	to	2.8	-2.3	-6.1	to	1.5	-2.3	-6.2	to	1.6
18 – 25 weeks' gestation	3.2	-0.6	to	7.0	1.1	-3.3	to	5.4	2.7	-1.8	to	7.2
> 25 weeks' gestation	-0.9	-4.5	to	2.7	-3.9	-8.1	to	0.4	-4.2	-8.6	to	0.2
Mean of three urines	0.6	-4.4	to	5.5	-2.5	-8.1	to	3.2				
Diethyl alkyl phosphates [d]												
< 18 weeks' gestation	0.1	-2.4	to	2.6	-2.0	-4.6	to	0.6	-2.2	-4.9	to	0.5
18 – 25 weeks' gestation	2.2	-0.4	to	4.7	1.1	-1.7	to	3.8	1.3	-1.5	to	4.2
> 25 weeks' gestation	2.2	-0.3	to	4.7	0.4	-2.9	to	3.7	0.4	-2.9	to	3.8
Mean of three urines	0.6	-4.4	to	5.5	1.4	-2.9	to	5.8				
Dimethyl alkyl phosphates [e]												
< 18 weeks' gestation	-1.7	-5.0	to	1.6	-2.6	-6.3	to	1.0	-2.6	-6.2	to	1.1
18 – 25 weeks' gestation	2.6	-1.0	to	6.3	0.5	-3.6	to	4.6	2.1	-2.2	to	6.4
> 25 weeks' gestation	-1.6	-5.1	to	1.8	-4.3	-8.3	to	-0.3	-4.5	-8.6	to	-0.4
Mean of three urines	0.6	-4.4	to	5.5	-3.1	-8.6	to	2.3				

a. Adjusted for child sex, maternal age, ethnicity (Dutch, other-western, and non-western), education (low, intermediate, and high), income (low, middle, and high), marital status, maternal non-verbal IQ, maternal alcohol consumption (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasional alcohol consumption during pregnancy, and frequent alcohol consumption during pregnancy), BMI categories (<18.5, 18.5-25, 25-30, and 30+), height of the mother, parity categories (0, 1, and 2+), and smoking categories (no smoking during pregnancy, smoked until pregnancy was known, and smoked during pregnancy).
b. Adjusted model with the inclusion of the three exposures in one model.
c. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.
d. Diethyl alkyl phosphates is the sum of DEDTP, DETP and DEP.
e. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP and DMP.

Table S24. Complete-case only model (with the inclusion of IT-Home score): Difference in child non-verbal IQ test score at age six years (and 95% confidence interval) per log10 nmol/g creatinine increase in maternal urine dialkyl phosphate metabolite concentration, by timing of pregnancy urine sampling, and degree of adjustment, among mother-child pairs with complete covariate data.

	Type of adjustment								
	None (n=708)			Adjusted (n=384) [a]			Mutually adjusted (n=384) [b]		
Dialkyl Phosphate Type	β	(95% CI)		β	(95% CI)		β	(95% CI)	
Dialkyl phosphates (total) [c]									
< 18 weeks' gestation	-0.7	-4.2 to 2.8		-0.5	-5.1 to 4.0		-1.0	-5.7 to 3.6	
18 – 25 weeks' gestation	3.2	-0.6 to 7.0		3.3	-1.7 to 8.4		4.4	-0.9 to 9.6	
> 25 weeks' gestation	-0.9	-4.5 to 2.7		-2.5	-7.4 to 2.4		-3.4	-8.4 to 1.6	
Mean of three urines	0.6	-4.4 to 5.5		0.9	-5.8 to 7.5				
Diethyl alkyl phosphates [d]									
< 18 weeks' gestation	0.1	-2.4 to 2.6		-1.7	-4.6 to 1.3		-1.9	-4.8 to 1.1	
18 – 25 weeks' gestation	2.2	-0.4 to 4.7		1.7	-1.5 to 4.8		1.9	-1.3 to 5.1	
> 25 weeks' gestation	2.2	-0.3 to 4.7		0.1	-3.7 to 4.0		-0.1	-4.0 to 3.8	
Mean of three urines	0.6	-4.4 to 5.5		0.9	-5.8 to 7.5				
Dimethyl alkyl phosphates [e]									
< 18 weeks' gestation	-1.7	-5.0 to 1.6		-1.1	-5.5 to 3.4		-1.3	-5.8 to 3.1	
18 – 25 weeks' gestation	2.6	-1.0 to 6.3		2.7	-2.2 to 7.5		3.6	-1.4 to 8.6	
> 25 weeks' gestation	-1.6	-5.1 to 1.8		-2.7	-7.3 to 2.0		-3.3	-8.1 to 1.5	
Mean of three urines	0.6	-4.4 to 5.5		0.9	-5.8 to 7.5				

a. Adjusted for child sex, maternal age, ethnicity (Dutch, other-western, and non-western), education (low, intermediate, and high), income (low, middle, and high), marital status, maternal non-verbal IQ, maternal alcohol consumption (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasional alcohol consumption during pregnancy, and frequent alcohol consumption during pregnancy), BMI categories (<18.5, 18.5-25, 25-30, and 30+), height of the mother, parity categories (0, 1, and 2+), IT-Home quartiles, and smoking categories (no smoking during pregnancy, smoked until pregnancy was known, and smoked during pregnancy).
b. Adjusted model with the inclusion of the three exposures in one model.
c. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.
d. Diethyl alkyl phosphates is the sum of DEDTP, DETP and DEP.
e. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP and DMP.

Table S25. Excluding DEDTP (i.e., including only those metabolites with at least 80% of values >LOD): Difference in child non-verbal IQ test score at age six years (and 95% confidence interval) per log10 nmol/g creatinine increase in maternal urine dialkyl phosphate metabolite concentration, by timing of pregnancy urine sampling and degree of adjustment (N=708).

	Type of adjustment								
	None			Adjusted [a]			Mutually adjusted [b]		
Dialkyl Phosphate Type	β	(95% CI)		β	(95% CI)		β	(95% CI)	
Dialkyl phosphates (total)									
< 18 weeks' gestation	-0.7	-4.2 to	2.8	-1.9	-5.3 to	1.5	-1.9	-5.3 to	1.6
18 – 25 weeks' gestation	3.2	-0.6 to	7.0	0.9	-2.9 to	4.7	2.6	-1.4 to	6.6
> 25 weeks' gestation	-0.8	-4.4 to	2.8	-3.9	-7.5 to	-0.3	-4.3	-8.1 to	-0.6
Mean of three urines	0.6	-4.3 to	5.5	-2.5	-7.4 to	2.4			
Diethyl alkyl phosphates [d]									
< 18 weeks' gestation	0.1	-2.4 to	2.6	-1.4	-3.9 to	1.0	-1.6	-4.1 to	0.9
18 – 25 weeks' gestation	2.1	-0.4 to	4.6	0.5	-2.0 to	3.0	0.9	-1.7 to	3.4
> 25 weeks' gestation	2.2	-0.3 to	4.7	-0.2	-2.8 to	2.3	-0.3	-2.8 to	2.3
Mean of three urines	4.4	0.6 to	8.2	1.0	-2.8 to	4.9			
Dimethyl alkyl phosphates [c]									
< 18 weeks' gestation	-1.6	-4.9 to	1.7	-2.5	-5.7 to	0.7	-2.3	-5.6 to	0.9
18 – 25 weeks' gestation	2.6	-1.0 to	6.3	0.7	-3.0 to	4.3	2.3	-1.4 to	6.1
> 25 weeks' gestation	-1.6	-5.0 to	1.9	-4.1	-7.5 to	-0.7	-4.3	-7.9 to	-0.8
Mean of three urines	-0.6	-5.4 to	4.1	-3.0	-7.7 to	1.7			

a. Adjusted for child sex, maternal age, ethnicity (Dutch, other-western, and non-western), education (low, intermediate, and high), income (low, middle, and high), marital status, maternal alcohol consumption (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasional alcohol consumption during pregnancy, and frequent alcohol consumption during pregnancy), maternal non-verbal IQ, BMI categories (<18.5, 18.5-25, 25-30, and 30+), height of the mother, parity categories (0, 1, and 2+), IT-Home quartiles, and smoking categories (no smoking during pregnancy, smoked until pregnancy was known, and smoked during pregnancy).

b. Adjusted model with the inclusion of the three exposures in one model.

c. Total dialkyl phosphates is the sum of DETP, DEP, DMDTP, DMTP and DMP.

d. Diethyl alkyl phosphates is the sum of DETP and DEP.

e. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP and DMP.

Table S26. Difference in Mosaics test score at age six years (and 95% confidence interval) per log10 nmol/g creatinine increase in maternal urine dialkyl phosphate metabolite concentration, by timing of pregnancy urine sampling and degree of adjustment (N=708).

	Type of adjustment								
	None			Adjusted [a]			Mutually adjusted [b]		
Dialkyl Phosphate Type	β	(95% CI)		β	(95% CI)		β	(95% CI)	
Dialkyl phosphates (total) [c]									
< 18 weeks' gestation	-1.3	-4.9	to 2.3	-3.1	-6.5	to 0.4	-3.0	-6.6	to 0.6
18 – 25 weeks' gestation	2.7	-1.2	to 6.7	0.1	-3.7	to 4.0	1.8	-2.3	to 5.9
> 25 weeks' gestation	0.2	-3.6	to 3.9	-3.3	-7.0	to 0.4	-3.3	-7.2	to 0.5
Mean of three urines	0.5	-4.7	to 5.6	-3.4	-8.5	to 1.6			
p homogeneity [d]									0.110
Diethyl alkyl phosphates [e]									
< 18 weeks' gestation	0.5	-2.1	to 3.2	-1.2	-3.7	to 1.3	-1.5	-4.1	to 1.1
18 – 25 weeks' gestation	2.8	0.1	to 5.4	1.0	-1.5	to 3.6	1.2	-1.5	to 3.8
> 25 weeks' gestation	3.7	1.0	to 6.3	0.9	-1.7	to 3.4	0.8	-1.9	to 3.4
Mean of three urines	5.7	1.7	to 9.6	1.6	-2.3	to 5.6			
p homogeneity [d]									0.457
Dimethyl alkyl phosphates [f]									
< 18 weeks' gestation	-2.2	-5.6	to 1.3	-3.6	-6.9	to -0.3	-3.4	-6.7	to 0.0
18 – 25 weeks' gestation	2.2	-1.6	to 6.1	0.0	-3.7	to 3.7	1.8	-2.0	to 5.7
> 25 weeks' gestation	-1.1	-4.7	to 2.5	-3.9	-7.4	to -0.4	-3.9	-7.6	to -0.3
Mean of three urines	-1.1	-6.0	to 3.9	-4.1	-9.0	to 0.7			
p homogeneity [d]									0.039

a. Adjusted for child sex, maternal age, ethnicity (Dutch, other-western and non-western), education (low, intermediate, and high), income (low, middle, and high), marital status, maternal alcohol consumption (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasional alcohol consumption during pregnancy, and frequent alcohol consumption during pregnancy), maternal non-verbal IQ, BMI categories (<18.5, 18.5-25, 25-30, and 30+), height of the mother, parity categories (0, 1, and 2+), IT-Home quartiles, and smoking categories (no smoking during pregnancy, smoked until pregnancy was known, and smoked during pregnancy).
b. Adjusted model with the inclusion of the three exposures in one model.
c. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.
d. Multiple-partial F test used to test whether exposure from different time points relates in the same manner to Mosaics scores.
e. Diethyl alkyl phosphates is the sum of DEDTP, DETP and DEP.
f. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP and DMP.

Table S27. Difference in Categories test score at age six years (and 95% confidence interval) per log10 nmol/g creatinine increase in maternal urine dialkyl phosphate metabolite concentration, by timing of pregnancy urine sampling and degree of adjustment (N=708).

	Type of adjustment								
	None			Adjusted [a]			Mutually adjusted [b]		
Dialkyl Phosphate Type	β	(95% CI)		β	(95% CI)		β	(95% CI)	
Dialkyl phosphates (total) [c]									
< 18 weeks' gestation	0.6	-2.9	to 4.1	0.6	-2.9	to 4.2	0.4	-3.3	to 4.1
18 – 25 weeks' gestation	3.0	-0.8	to 6.8	2.4	-1.5	to 6.3	3.3	-0.9	to 7.4
> 25 weeks' gestation	-1.4	-4.9	to 2.2	-2.4	-6.1	to 1.4	-3.3	-7.2	to 0.6
Mean of three urines	1.2	-3.7	to 6.1	0.9	-4.2	to 6.0			
p homogeneity [d]									0.128
Diethyl alkyl phosphates [e]									
< 18 weeks' gestation	-0.5	-3.0	to 2.1	-1.0	-3.6	to 1.5	-1.1	-3.7	to 1.5
18 – 25 weeks' gestation	1.1	-1.5	to 3.6	0.4	-2.2	to 3.0	0.8	-1.9	to 3.5
> 25 weeks' gestation	0.0	-2.6	to 2.5	-0.9	-3.6	to 1.7	-1.0	-3.7	to 1.7
Mean of three urines	1.7	-2.2	to 5.5	0.6	-3.4	to 4.6			
p homogeneity [d]									0.520
Dimethyl alkyl phosphates [f]									
< 18 weeks' gestation	-0.1	-3.4	to 3.2	0.2	-3.2	to 3.5	0.1	-3.4	to 3.5
18 – 25 weeks' gestation	2.6	-1.0	to 6.3	2.0	-1.8	to 5.8	2.8	-1.2	to 6.7
> 25 weeks' gestation	-1.3	-4.8	to 2.1	-2.1	-5.7	to 1.5	-2.8	-6.5	to 0.9
Mean of three urines	0.8	-3.9	to 5.5	0.8	-4.1	to 5.7			
p homogeneity [d]									0.200

a. Adjusted for child sex, maternal age, ethnicity (Dutch, other-western and non-western), education (low, intermediate, and high), income (low, middle, and high), marital status, maternal alcohol consumption (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasional alcohol consumption during pregnancy, and frequent alcohol consumption during pregnancy), maternal non-verbal IQ, BMI categories (<18.5, 18.5-25, 25-30, and 30+), height of the mother, parity categories (0, 1, and 2+), IT-Home quartiles, and smoking categories (no smoking during pregnancy, smoked until pregnancy was known, and smoked during pregnancy).

b. Adjusted model with the inclusion of the three exposures in one model.

c. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.

d. Multiple-partial F test used to test whether exposure from different time points relates in the same manner to Categories scores.

e. Diethyl alkyl phosphates is the sum of DEDTP, DETP and DEP.

f. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP and DMP.

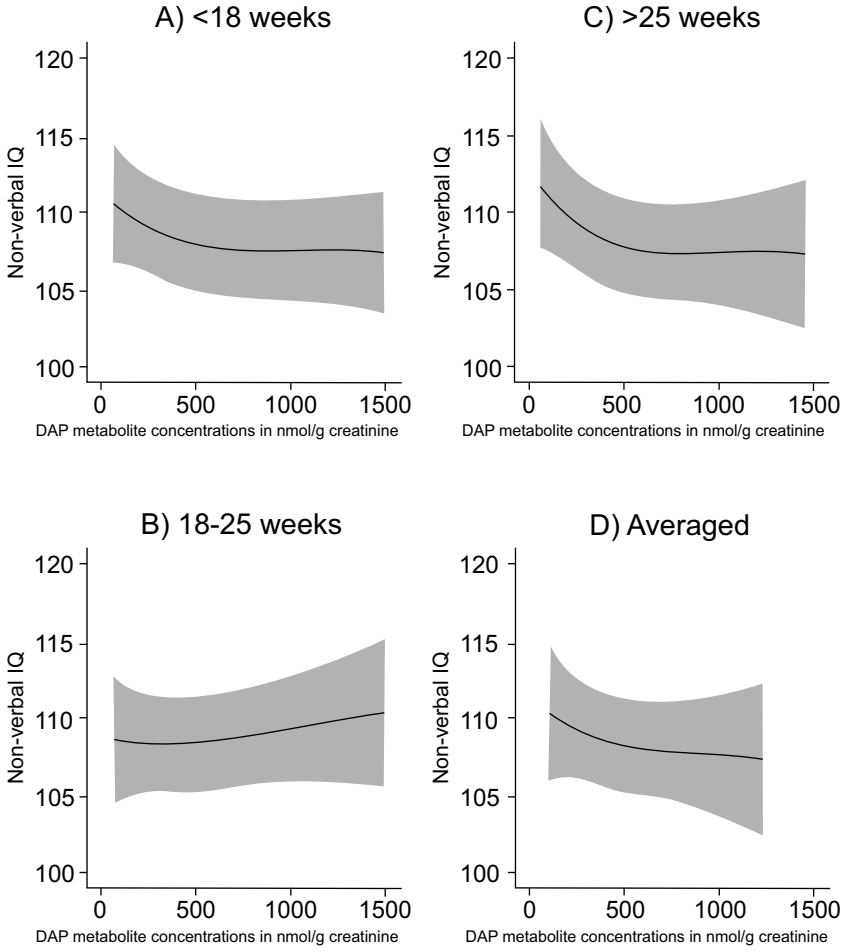

Figure S1. Restricted cubic spline (and 95% confidence interval) of adjusted child non-verbal IQ scores and (untransformed) total dialkyl phosphate concentrations, by timing of pregnancy urine sampling (n=708). Adjusted for child sex, maternal age, ethnicity (Dutch, other-western, and non-western), education (low, intermediate, and high), income (low, middle, and high), marital status, maternal alcohol consumption (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasional alcohol consumption during pregnancy, and frequent alcohol consumption during pregnancy), maternal non-verbal IQ, BMI categories (<18.5, 18.5-25, 25-30, and 30+), height of the mother, parity categories (0, 1, and 2+), IT-Home quartiles, and smoking categories (no smoking during pregnancy, smoked until pregnancy was known, and smoked during pregnancy).

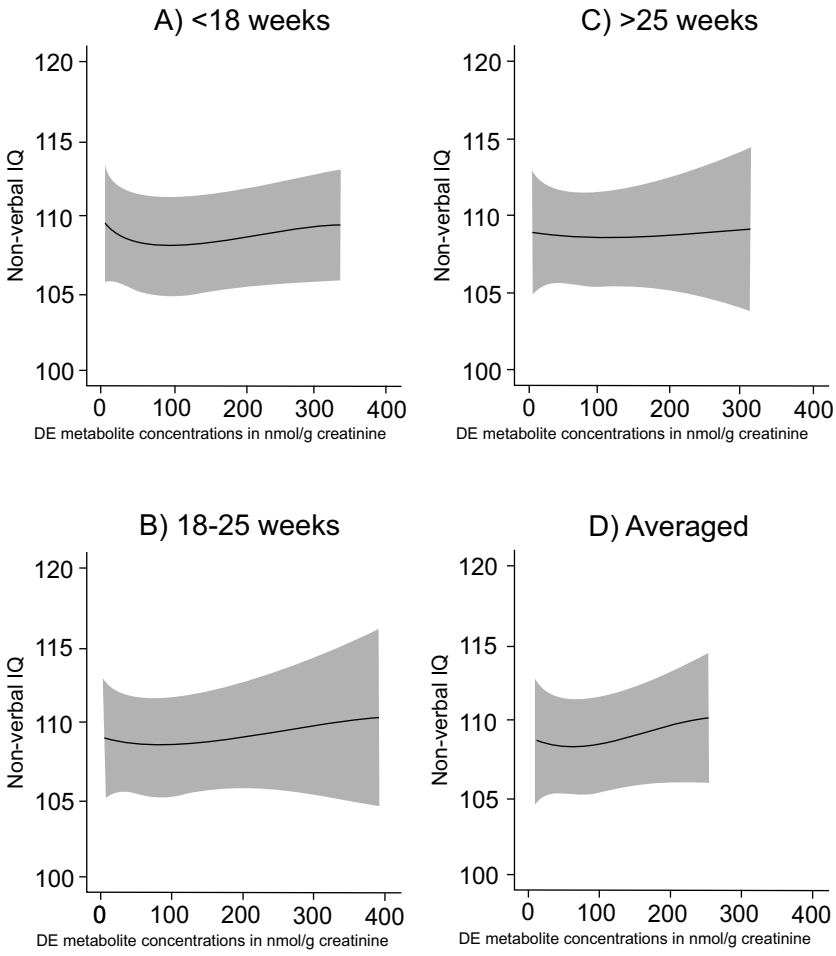

Figure S2. Restricted cubic spline (and 95% confidence interval) of adjusted child non-verbal IQ scores and (untransformed) diethyl alkyl phosphate concentrations, by timing of pregnancy urine sampling (n=708). Adjusted for child sex, maternal age, ethnicity (Dutch, other-western and non-western), education (low, intermediate, and high), income (low, middle, and high), marital status, maternal alcohol consumption (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasional alcohol consumption during pregnancy, and frequent alcohol consumption during pregnancy), maternal non-verbal IQ, BMI categories (<18.5, 18.5-25, 25-30, and 30+), height of the mother, parity categories (0, 1, and 2+), IT-Home quartiles, and smoking categories (no smoking during pregnancy, smoked until pregnancy was known, and smoked during pregnancy).

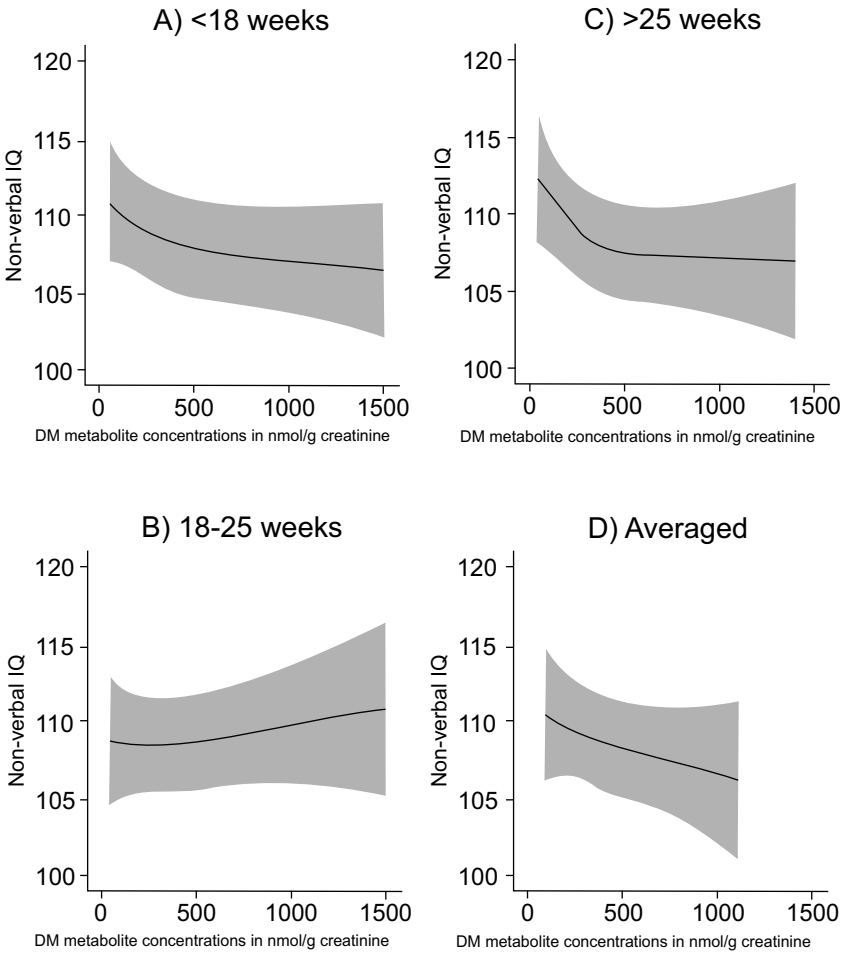

Figure S3. Restricted cubic spline (and 95% confidence interval) of adjusted child non-verbal IQ scores and (untransformed) dimethyl alkyl phosphate concentrations, by timing of pregnancy urine sampling (n=708). Adjusted for child sex, maternal age, ethnicity (Dutch, other-western and non-western), education (low, intermediate, and high), income (low, middle, and high), marital status, maternal alcohol consumption (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasional alcohol consumption during pregnancy, and frequent alcohol consumption during pregnancy), maternal non-verbal IQ, BMI categories (<18.5, 18.5-25, 25-30, and 30+), height of the mother, parity categories (0, 1, and 2+), IT-Home quartiles, and smoking categories (no smoking during pregnancy, smoked until pregnancy was known, and smoked during pregnancy).

5

Chapter 5

Organophosphate pesticide metabolite concentrations in urine during pregnancy and offspring attention-deficit hyperactivity disorder and autistic traits

van den Dries, M. A., Guxens, M., Pronk, A., Spaan, S., El Marroun, H., Jusko, T. A., Longnecker, M. P., Ferguson, K. K., & Tiemeier, H. (2019).

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Abstract

Background: Prenatal exposure to organophosphate (OP) pesticides has been associated with altered neuronal cell development and behavioral changes in animal offspring. However, the few studies investigating the association between prenatal OP pesticide exposure and neurodevelopmental outcomes such as Attention-Deficit Hyperactivity Disorder (ADHD) and autistic traits in children produced mixed findings.

Objectives: The objective of the present study was to examine whether maternal urinary concentrations of OP pesticide metabolites are associated with ADHD and autistic traits in children participating in the Generation R Study, a population-based birth cohort from Rotterdam, the Netherlands.

Method: Maternal concentrations of 6 dialkylphosphates (DAPs) were measured using gas chromatography coupled with tandem mass spectrometry in urine samples collected at <18 weeks, 18-25 weeks, and > 25 weeks of gestation in 784 mother-child pairs. DAP metabolite concentrations were expressed as molar concentrations divided by creatinine levels and log10 transformed. ADHD traits were measured at ages 3, 6, and 10 years using the Child Behavior Checklist (CBCL) (n = 781) and autistic traits were measured at age 6 years using the Social Responsiveness Scale (SRS) (n = 622). First, regression models were fit for the averaged prenatal exposure across pregnancy. Second, we investigated associations for each collection phase separately, and applied a mutually adjusted model in which the effect of prenatal DAP concentrations from each time period on ADHD and autistic traits were jointly estimated. All associations were adjusted for relevant confounders.

Results: Median DAP metabolite concentration was 309 nmol/g creatinine at <18 weeks, 316 nmol/g creatinine at 18–25 weeks, and 308 nmol/g creatinine at >25 weeks of gestation. Overall, DAP metabolite concentrations were not associated with ADHD traits. For instance, a log10 increase in averaged total DAP concentrations across gestation was not associated with a lower ADHD score (-0.03 per SD 95%CI: −0.28 to 0.23). Similarly, no associations between maternal DAP concentrations and autistic traits were detected.

Conclusions: In this study of maternal urinary DAP metabolite concentrations during pregnancy, we did not observe associations with ADHD and autistic traits in children. These are important null observations because of the relatively high background DAP concentrations across pregnancy, the relatively large sample size, and the 10-year follow-up of the offspring. Given the measurement error inherent in our OP pesticide exposure biomarkers, future studies using more urine samples are needed to accurately measure OP pesticide exposure over pregnancy in relation to ADHD and autistic traits.

Introduction

Organophosphate (OP) pesticides are a class of insecticides commonly used in agriculture. Some of the active OP pesticides may remain on or in food after they are applied to food crops[1] and the exposure of non-occupationally exposed individuals occurs most likely through their diet.[2-5] After ingestion, most OP pesticides undergo bioactivation, during which the toxic oxon form is established, followed by detoxification, which produces up to 6 non-specific dialkyl phosphate (DAP) metabolites.[6] Preformed DAP metabolites also exist in the food supply.[7-9] It is therefore uncertain to what degree total DAP metabolite concentrations reflect actual OP pesticide exposure or the ingestion of possibly less toxic DAP metabolites.[10] However, the estimation of urinary DAP metabolite concentrations is considered a non-invasive and useful biomarker for OP pesticide exposure,[11] and thus, the most-used method of estimating exposure to this class of compounds in general populations.[12]

High OP pesticide exposure is neurotoxic for both animals and humans.[13-15] However, both animal and human studies have suggested that even low-dose OP pesticide exposure may have negative health consequences.[16] Animal studies investigating exposure to the OP pesticides chlorpyrifos, diazinon and malathion (of which the residues were frequently being detected on fruit and vegetables between 2004 and 2006 in the Netherlands)[17] have shown that exposure levels below the threshold for acetylcholinesterase inhibition can induce changes in neurochemistry and behavior,[18] result in cognitive impairments[19,20] and change the expression of genes related to mental disorders.[21] Low-dose OP pesticide exposure in animal studies also changed neuronal cell development,[22] induced oxidative stress,[23,24] and affected the thyroid and the reproductive systems.[25-27] Moreover, animals prenatally exposed to these OP pesticides had higher activity rates, greater motor agitation and hyperactivity signs, lower level of social behavior, and animals were limited in their exploration of novel objects.[28,29]

Because the human brain is particularly susceptible to neurotoxicity during fetal life,[30] and because OP pesticides can cross the placental barrier and the blood-brain barrier,[31] most epidemiological studies of low-dose OP pesticide exposure focus on prenatal exposure in relation to neurodevelopment.[32] Several of these studies have found prenatal OP pesticide exposure to be associated with or suggestive of poorer reflexes in neonates,[33,34] mental and psychomotor developmental delays in the offspring aged 1 to 3 years,[35-37] and decreased intellectual functioning in children aged 6 to 9 years[35,38-40] Yet, other studies have not observed associations with neurodevelopmental outcomes.[32] For example, Cartier et al. (2016) did not find evidence for an association between prenatal OP pesticide exposure and intellectual functioning in children aged 6 years.

Few studies have explored the association between OP pesticide exposure and Attention-Deficit Hyperactivity Disorder (ADHD) and autistic traits in children, and have reported inconsistent findings. A prospective birth cohort study among ethnic minorities from inner-city New York observed associations between prenatal OP pesticide exposure and ADHD traits in 228 children aged 3 years.[37] Also, another birth cohort study among low-income participants from farmworking communities in California observed associations with ADHD traits in 322 children aged 5 years.[41] Yet, Eskenazi et al. (2007) did not observe these associations among children aged 2 years using data from the same cohort as Marks et al. (2010). These cohort studies were also used to assess the association between prenatal OP pesticide exposure and pervasive developmental disorder (PDD), which includes Autism Spectrum Disorders (ASDs) and found prenatal exposure to OP pesticides to be predictive of PDD at 2 to 3 years,[36,37] but the number of PDD cases was small in one of the studies.[37] Next, a study using data from the same cohort as Eskenazi et al. (2007) and Marks et al. (2010) found that children prenatally exposed to higher levels of OP pesticides had more autistic traits as measured with the Social Responsiveness Scale (SRS) in 246 children aged 14 years.[42] Yet, another study using data from 224 mother-child pairs from a metropolitan area in Ohio found that prenatal OP pesticide exposure did not increase autistic symptoms at age 8 years.[43] Only in subgroups, Furlong et al. (2014) observed an association between OP pesticide exposure and autistic symptoms among Black (n = 42) and male children (n = 66) using 136 mother-child pairs from New York. Similarly, Philippat et al. (2018), using data from a cohort study of women at high risk for having a child with ASD, did not observe an overall association among 203 children aged 3 years. However, after stratifying by sex prenatal OP pesticide exposure was associated with an increased risk of ASD among girls (n = 78).[44]

This heterogeneity may be explained by differences in study areas and study populations. For example, 3 studies took place in California in a farmworker community where the use of insecticides is abundant,[36,41,42] whereas other studies took place in urban areas[37,43,45] where the source and route of OP pesticide exposure may be different. Next, several of these studies were small in sample size. This may have reduced the power to detect associations and perform interaction analyses. Also, most of these studies included 1 or 2 urine specimens per subject to measure OP pesticide exposure. Analyzing multiple urine specimens per subject is of importance, because the urinary concentration of DAP metabolites reflects only recent exposure, and individual exposure differs substantially from day-to-day, depending on diet.[4,46]

Therefore, much uncertainty still exists about the relationship between fetal exposure to OP pesticides and the development of ADHD and ASD. The Generation R Study provides suitable data to address these research gaps because of the large sample size, 3 repeated measurements of maternal urinary concentrations of OP pesticide metabolites, repeated

neurobehavioral measurements, and the availability of detailed demographic information. Furthermore, as documented elsewhere, the median total DAP concentrations among the Generation R Study mothers was more than 2-fold higher compared with background-exposed pregnant women in the U.S living in non-agricultural communities, which suggests a greater range of exposure and thus statistical power with which to evaluate exposure—disease associations.[47] The objective of the present study was to examine whether maternal urinary concentrations of OP pesticide metabolites is related to ADHD and autistic traits in young children.

Methods

Study population and follow-up

Generation R is a prospective population-based birth cohort designed to identify early environmental and genetic determinants of development.[48] Briefly, all mothers who resided in the study area in Rotterdam, the Netherlands, and had a delivery date between April 2002 and January 2006 were eligible. Mothers were enrolled during pregnancy or in the first months after the birth of their child when newborns attended child health centers for routine visits. The study protocol underwent human subjects review at Erasmus Medical Center, Rotterdam, the Netherlands (IRB Registration no.: IRB00001482, MEC 198.782.2001.31, MEC 217.595/2002/202, MEC-2007-413, MEC-2012-165). Mothers provided written informed consent for themselves and their children.

Among the 9778 mothers who participated in the study, 8879 (91%) were enrolled during, as opposed to after, pregnancy. Between February 2004 and January 2006, spot urine specimens during early, middle, and late pregnancy (<18, 18–25, >25 weeks of gestational age, respectively) were collected at the time of routine ultrasound examinations when in total, 4918 women were enrolled. Of these, 2083 women provided a complete set of 3 urine specimens. From birth until the age of 4 years, data collection was performed by mailed questionnaires and by routine child health center visits. At child age 6 and 10 years, families were invited to participate in an in-person follow-up to collect neurobehavioral data, additional biospecimens, and sociodemographic and health data. We selected samples based on follow-up data with relevant outcomes, which was obtained for 1449 children of the 2083 women with a complete set of urine specimens. The availability of follow-up data permitted studies of prenatal OP pesticide exposure and child health, including neurodevelopment. From these 1449, 800 mother-child pairs were selected at random for lab analyses of DAP metabolites in the maternal and child urine samples. Of those, 784 had a sufficient volume of urine for analyses. The final analytic sample included 781 mother-child pairs with exposure and data on ADHD traits (from age 3 to 10 years), and 622 mother-child pairs with exposure and data on autistic traits (at age 6 years).

Urine collection and analysis of DAP metabolites

Maternal spot urine specimens were collected during early, mid-, and late pregnancy (<18, 18–25, >25 weeks of gestational age, respectively). Child spot urine specimens were collected when mother-child pairs attended the 6-year examination. Details of urine specimen collection have been described elsewhere.[49] All urine samples were collected between 8 am and 8 pm in 100 ml polypropylene urine collection containers that were kept for a maximum of 20 h in a cold room (4 °C) before being frozen at –20 °C in 20 ml portions in 25 ml polypropylene vials. Measurements of 6 non-specific DAP metabolites of OP pesticides were conducted at Institut National de Santé Publique (INSPQ) in Quebec, Canada, using gas chromatography coupled with tandem mass spectrometry (GC–MS/MS).[50] Three dimethyl (DM) metabolites (dimethylphosphate (DMP), dimethylthiophosphate (DMTP), and dimethyldithiophosphate (DMDTP)) were determined, as well as 3 diethyl (DE) metabolites (diethylphosphate (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP)). The limit of detection (LOD) was 0.26 µg/l for DMP (0% < LOD), 0.40 for DMTP (2–4% < LOD), 0.09 for DMDTP (18–20% < LOD), 0.50 for DEP (3–5% < LOD), 0.12 for DETP (12% < LOD) and 0.06 for DEDTP (81–85% < LOD). Measured concentrations below the LOD were included in the data analysis. The inter-day precision of the method during this project, expressed as the coefficient of variation (CV%), varied between 4.2 and 8.8 for DEDTP, 4.1–7.2 for DEP, 5.0–9.1 for DETP, 5.5–7.1 for DMDTP, 5.3–8.0 for DMP and 5.5–7.7 for DMTP based on reference materials (clinical check-urine level II 637 E-495 and MRM E-459).[5] Molar concentrations were used to compare our results with those from other studies, based on the following molecular weights: DMP 126.0, DMTP 142.1, DMDTP 158.2, DEP 154.1, DETP 170.2, and DEDTP 186.2 g/mol. To account for urinary dilution, creatinine concentrations were determined based on the Jaffe reaction.[51,52] The LOD for creatinine was 0.28 mmol/l, and the day-to-day CV% varied between 3.0 and 3.3.[5]

Assessment of child ADHD traits

Child emotional and behavioral problems were assessed by maternal report with the Child Behavior Checklist (CBCL) 1.5–5[53] during the assessments at child age 3, and 6 years, and with the CBCL 6–18 at child age 10 years.[54] The CBCL is an internationally validated and reliable measure of emotional and behavioral problems.[54] The CBCL measures emotional and behavioral problems on a continuous severity scale and research has shown that symptom scores predict psychiatric disorders as defined by the DSM in adulthood.[55,56] Each item (CBCL 1.5–5: 99 items, CBCL 6–18: 112 items) within different scales (CBCL 1.5–5: 7 scales, CBCL 6–18: 8 scales) is scored on a 3 point rating scale 0 = 'not true', 1 = 'somewhat or sometimes true', and 2 = 'very true or often true', based on the preceding 2 months for the CBCL 1.5–5 and the preceding 6 months for the CBCL 6–18. The scales were found to be generalizable across 23 countries, including

the Netherlands.[57] From the CBCL checklist, we used the standardized sum score of the Diagnostic and Statistical Manual of Mental Disorders (DSM)-oriented ADHD traits across childhood for our analyses. The sum scores of ADHD traits were standardized to make the CBCL 1.5–5 (6 items) and the CBCL 6–18 (7 items) comparable, with a higher score indicating a higher level of ADHD traits.

Assessment of child autistic traits

At age 6 years, the SRS was administered to obtain a measure of autistic traits.[58] The SRS provides a valid quantitative measure of subclinical and clinical autistic traits and assesses various dimensions of interpersonal behavior, communication and repetitive/stereotypic behavior characteristic of ASD.[59] The SRS represents the mothers' observation of the child's social behavior during the previous 6 months. The SRS is a useful screening tool to identify children who need further ASD-specific diagnostic assessment. The SRS has excellent correspondence to ASD classification according to the Developmental, Dimensional, and Diagnostic interview (3Di) and the Autism Diagnostic Observation Schedule (ADOS).[60] We used an abbreviated version of the SRS with a total of 18 items[61] to reduce participant burden. These items cover 3 domains: social cognition, social communication, and autistic mannerism. Previous studies have shown high correlations ($r > 0.90$) between the total scores of the abbreviated version of the SRS and the complete version of the SRS.[62] We used the SRS total score as a continuous measure in our analyses.

Additional data collection

Maternal reproductive, sociodemographic, and cognitive data were assessed by multiple questionnaires and instruments throughout the study. During pregnancy, data on maternal height and weight were collected, as was information on maternal age, maternal psychopathology (0= no problems, 1 = borderline: GSI score > 80%),[63] parity (0, 1, or 2+), smoking (no smoking during pregnancy, smoked until pregnancy recognized, and continued smoking during pregnancy), alcohol intake during pregnancy (no alcohol consumption during pregnancy, alcohol consumption until pregnancy recognized, continued occasionally (<1 glass/week), and continued frequently (1+ glass/week)), marital status (married/partner or single), household total net income (<1200 euro per month (i.e., below the Dutch social security level), 1200–2000 euro per month, >2000 euro per month), highest completed education level (low: <3 years of high school; intermediate: 3+ years of secondary education; and, high: university degree or higher vocational training), and ethnicity (Dutch national origin, other-Western and non-Western). Further, body mass index (BMI) was calculated and categorized into 4 groups (<18.5, 18.5–<25, 25–<30, and ≥30).

After birth, mothers reported on the duration of breastfeeding by postal questionnaire when the child was 2 months, 6 months and 12 months old. Mothers were asked whether

they ever breastfed their child, and if yes, duration of any breastfeeding was assessed by asking at what age of the infant they stopped breastfeeding (in months). Next, an adapted Infant/Toddler Home Observation for Measurement of the Environment (IT-HOME) inventory[64] was administered during a home visit at approximately 3 months of age (SD = 1.17 months). The validated 29-item version of the IT-HOME was used to measure the events, objects, and social interactions experienced by the child in the family context.[65] Higher scores on the IT-HOME indicate a more enriched environment. Maternal IQ was measured when mother-child pairs attended the 6-year examination, and was assessed using a computerized Ravens Advanced Progressive Matrices Test, set I.[66] The test is a 12-item reliable and validated short version of the Raven's Progressive Matrices to assess non-verbal cognitive ability.[67]

Statistical methods

The 3 DM metabolites (DMP, DMTP, and DMDTP) were summed as total DM (nmol/l) and the 3 DE metabolites (DEP, DETP, and DEDTP) were summed as total DE (nmol/l). Total DAP concentrations (nmol/l) were calculated by summing the 6 metabolites. Urinary concentrations were expressed on a volume and creatinine basis (nmol/g creatinine) and log10 transformed. For DAP metabolites, a small number of concentrations were missing due to insufficient samples or machine errors (≤5 measurements for any visit for DMs; ≤23 for DEs; ≤5 per visit for creatinine). Missing DAP metabolite values and missing covariate data were 10 times imputed with the Multivariate Imputation by Chained Equations (MICE) method in R.[68,69] DAP metabolite concentrations were log10 transformed prior to the multiple imputation (MI) procedure to approach normality. Both ADHD traits scores and autistic traits scores were included as predictors for the imputation of covariates, but were not imputed.

To address our primary research objective, we created linear mixed effects models (LMM) using averaged DM, DE, and DAP concentrations over pregnancy in relation to repeated measures of ADHD and used linear regression models to assess the association between averaged DM, DE, and DAP concentrations and autistic traits. Because urinary DAP levels are highly variable over time, this average is likely a better estimate of each participant's exposure than any single exposure measurement.[70] As a secondary approach, we investigated the DAP – ADHD traits and the DAP–autistic traits association for each collection phase (gestational age <18 weeks, 18–25 weeks, and >25 weeks) separately, and applied a mutually adjusted model in which the effect of prenatal DAP concentration from each time period on ADHD and autistic traits was jointly estimated. This additional approach was chosen to identify possible windows of vulnerability and to be able to compare our results with other studies that used a single spot urine sample in pregnancy to determine OP pesticide exposure.

Each LMM included a random intercept, a random slope of time (the age of the child at the outcome ascertainment in years), and an Autoregressive (order 1) covariance structure which improved the model fit based on a lower Akaike information criteria (AIC). The inclusion of an interaction term between time and exposure did not improve the model AIC significantly. For the DAP–autistic traits analyses the autistic traits score was square root transformed to approach normality of the residuals.

All analyses consisted of an unadjusted model and an adjusted model. The adjustment variables were maternal age, psychopathology score, ethnicity, education, income, marital status, alcohol consumption during pregnancy, non-verbal IQ, BMI, height, parity, smoking during pregnancy, and child sex. Potential adjustment variables were selected a priori defined with a Directed Acyclic Graph (DAG) using the Dagitty software.[71] The DAG was based on previous studies of OP pesticide exposure and child neurodevelopment and on biologically plausible covariate–exposure and covariate–outcome associations observed in our data (See Supplementary Fig. S1). Additionally, adjusting for the possible confounders breastfeeding in months and IT-HOME score did not change the effect estimates meaningfully and were not included in the models.

Sensitivity analyses

Several sensitivity analyses were performed. First, potential effect modification by sex was explored via interaction terms, stratification, and augmented product terms,[72] because other studies have reported sex specific effects of the association between prenatal DAP metabolite concentrations with ADHD and autistic traits.[41,44,45] Second, we refit models with using dichotomous ADHD and autistic traits scores because several studies investigated the association between DAP metabolite concentrations with the use of clinical cases.[36,73] The dichotomization was based on the borderline clinical cut-off score for ADHD (>93rd percentile)[53,54] and SRS cut-off value for screening in the population (consistent with weighted scores of ≥1.078 for boys and ≥1.000 for girls),[59] and based on the >80th percentile of the ADHD and autistic traits scores (to increase power). Third, we used inverse probability weighting to correct for loss to follow-up and to account for potential selection bias because participants in our study sample were more likely be Dutch, older, have a higher level of education, and a higher income compared with the full cohort (Table S1). Fourth, we substituted values below the LOD with LOD/$\sqrt{2}$ rather than the use of the measured concentrations below the LOD, which were included in the primary analysis. The replacement of values below the LOD with LOD/$\sqrt{2}$ is a common substitution method in environmental exposure studies.[74] Fifth, we refit models with metabolite concentrations expressed as nmol/l with creatinine concentration added as a separate covariate which is another common method to adjust for creatinine concentrations. Sixth, because DAP metabolite concentrations demonstrated only weak to moderate reliability over pregnancy (e.g., intraclass correlation coefficient (ICC) for

DAP metabolites = 0.30),[70] we examined the effect of adjusting for measurement error by applying regression calibration.[75] Seventh, because preformed DAP metabolites may exist on fruits, and fruit intake is associated with DAP metabolite concentrations[5] in our study population, we additionally stratified the main analyses for maternal fruit intake (assessed using a modified version of a validated semiquantitative food frequency questionnaire.[76] The stratification was based on dichotomizing intake at the first quantile (75 g per day). Eight, we also adjusted for season of urine collection because there may be seasonal variation in food consumption and OP pesticides use which could affect DAP concentrations.[77] Ninth, since previous studies have suggested that families with low social economic status (SES) are more vulnerable to OP pesticide exposure,[78-80] we explored potential effect modification by education as a proxy of SES. Finally, we explored potential associations of child DAP metabolite concentrations with ADHD and autistic traits and investigated potential effect modification by sex, since few studies have reported these associations.[81,82] The child-DAP analyses were adjusted for maternal education, maternal age, maternal smoking, averaged prenatal DAP metabolite concentrations across pregnancy, child sex, child BMI at age 6 years, child ethnicity, household income at child age 6 years, and marital status of the mother at child age 6 years.

Results

Sample characteristics

Table 1 presents the maternal and infant characteristics at time of enrollment. The age at enrollment of the mothers participating in this study averaged 31 years (sd=5 years). Women included in the present analysis were older, had lower BMIs, nulliparous, Dutch, highly educated, married, occasional consumers of alcoholic beverages during pregnancy, less likely to smoke, and had higher incomes and lower maternal psychopathology scores compared with those not included.

DAP concentrations

Total DAP metabolites comprised mostly DM metabolites, and the median concentrations were fairly similar across the 3 sampling periods (Table 2). The total DAP metabolite concentrations measured between 18-25 weeks of gestation (median = 316 nmol/g creatinine) was slightly higher compared with the DAP metabolite concentrations measured at <18 weeks of gestation (median = 309 nmol/g creatinine) and > 25 weeks of gestation (median = 308 nmol/g creatinine). The ICC (estimated by using a 2-way mixed-effects model with absolute-agreement) for DAP metabolite concentrations varied between 0.22 and 0.26 for a single-measurement and 0.51 and 0.54 for the mean of the 3 measurements (Table S2). Moreover, the DAP metabolite concentrations across pregnancy showed weak correlations (r = 0.18–0.35) (Table S3).

Table 1. Characteristics of all participants of the Generation R Study and of the participants included in the analysis.

Characteristic	Generation R cohort (n=9778) [c]	Included in the ADHD analyses [a] (n=781) [c]	Included in the autistic traits analyses [b] (N=622) [c]
Infant characteristics			
Sex of infant at birth			
Male	50.6 %	50.8 %	51.4 %
Female	49.4 %	49.2 %	48.6 %
Missing, n	153	-	
Mother characteristics			
Age in years			
< 20	4.2 %	1.8 %	1.1 %
20-< 25	15.9 %	10.0 %	7.4 %
25-< 30	26.4 %	26.6 %	25.6 %
30-< 35	36.9 %	46.0 %	49.0 %
≥ 35	16.6	15.7 %	16.9
Missing, n	-	-	-
BMI			
< 18.5	2.1 %	2.3 %	2.4 %
18.5-< 25	57.9 %	65.9 %	67.3 %
25-< 30	26.3 %	23.5 %	22.3 %
≥ 30	13.8 %	8.3 %	8.1 %
Missing, n	899	4	2
Height in cm (quartiles)			
< 161	23.6 %	15.7 %	13.0 %
161 – < 168	27.4 %	30.6 %	29.1 %
168 – < 173	24.6 %	26.2 %	27.9 %
≥ 173	24.4 %	27.5 %	30.0 %
Missing, n	934	1	1
Parity (Previous births)			
0	55.1 %	62.4 %	63.5 %
1	30.2 %	26.6 %	26.0 %
≥ 2	14.7 %	11.0 %	10.5 %
Missing, n	378	4	2
Ethnicity			
Dutch	50.0 %	57.6 %	63.3 %
Other Western	11.6 %	8.9 %	9.0 %
Non-Western	38.4 %	33.5 %	27.7 %
Missing, n	694	-	-
Education [d]			
Low	26.5 %	14.8 %	11.9 %
Intermediate	30.7 %	30.2 %	27.8 %
High	42.8 %	55.0 %	60.2 %
Missing, n	1221	25	11
Household income in euro's			
<1200 per month	20.7 %	12.6 %	9.1 %
1200–2000 per month	18.5 %	16.6 %	15.5 %
> 2000 per month	60.8 %	70.8 %	75.4 %
Missing, n	3066	101	62
Marital status			
Married/ living with partner	85.5 %	89.8 %	91.4 %
No partner	14.5 %	10.2 %	8.6 %
Missing, n	1213	29	18
Non-verbal IQ score			
≤ 85	29.7 %	17.9 %	15.7 %
>85-≤ 100	25.7 %	22.8 %	23.3 %
>100 -< 115	34.7 %	31.2 %	31.3 %
≥ 115	19.8 %	28.0 %	29.7 %
Missing, n	5430	20	171

Continue

Continued

Characteristic	Generation R cohort (n=9778) [c]	Included in the ADHD analyses [a] (n=781) [c]	Included in the autistic traits analyses [b] (N=622) [c]
psychopathology (quartiles)			
< 0.08	24.1 %	24.9 %	26.4 %
0.08–< 0.17	25.2 %	29.7 %	29.6 %
0.17–> 0.38	25.7 %	24.4 %	24.9 %
≥ 0.38	25.0 %	21.1 %	19.1 %
Missing, n	3128	95	72
Smoking			
No smoking during pregnancy	73.4 %	77.1 %	79.7 %
Until pregnancy recognized	8.6 %	8.9 %	9.1 %
Continued during pregnancy	18.0 %	14.0 %	11.2 %
Missing, n	1534	63	51
Alcohol Beverage Consumption			
No alcohol consumption during pregnancy	48.0 %	36.7 %	33.7 %
Until pregnancy recognized	13.2 %	17.5 %	17.2 %
Continued occasionally (less than 1 glass/week)	31.6 %	39.3 %	42.0 %
Continued frequently (1 or more glass/ week for at least two trimesters)	7.2 %	6.5 %	7.1 %
Missing, n	1870	40	29

a. ADHD traits was measured at child mean age three, six and ten years.
b. Autistic traits was measured at child mean age six years.
c. Values shown are percentages.
d. Low: no education finished, Primary education, lower vocational training, intermediate general school or <3 years at general. Intermediate: ≥3 years of secondary education, Intermediate vocational training or first year of higher vocational training. High: university degree or higher vocational training.

Table 2. Descriptive statistics of maternal urinary dialkyl phosphate metabolite concentrations of 781 mothers participating in this study.

	nmol/g creatinine					nmol/l				
	min	p25	p50	p75	max	min	p25	p50	p75	max
Dialkyl phosphates in nmol/g creatinine (total) [a]										
< 18 weeks' gestation	15.4	188.1	309.4	499.3	6444.5	6.3	124.8	219.0	421.2	7798.7
18 – 25 weeks' gestation	41.0	206.8	316.1	485.9	3069.5	10.0	120.0	227.2	407.2	4607.7
> 25 weeks' gestation	21.0	194.1	308.0	489.3	3013.3	10.5	121.6	223.8	408.2	3332.6
Diethyl alkyl phosphates in nmol/g creatinine [b]										
< 18 weeks' gestation	0.0	25.0	43.1	79.4	3030.5	0.0	15.5	31.3	65.1	6818.6
18 – 25 weeks' gestation	0.0	23.3	41.6	74.5	660.5	0.0	12.6	27.9	57.0	1093.4
> 25 weeks' gestation	0.0	21.6	41.7	77.3	745.1	0.0	14.4	30.7	62.2	593.2
Dimethyl alkyl phosphates in nmol/g creatinine [c]										
< 18 weeks' gestation	6.7	148.6	242.4	416.1	6106.5	0.9	100.1	182.4	344.7	4221.0
18 – 25 weeks' gestation	24.8	169.4	268.6	415.4	2612.0	7.6	98.9	185.6	335.9	3902.2
> 25 weeks' gestation	12.2	157.1	248.3	398.9	2908.1	8.5	99.5	184.2	333.0	3300.5

a. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.
b. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP and DMP.
c. Diethyl alkyl phosphates is the sum of DEDTP, DETP and DEP.

ADHD and autistic traits descriptive statistics

Table 3 presents the descriptive statistics of the ADHD and autistic traits score. The mean ADHD score measured at age 3 years (m = 3.1, sd = 2.3) and at 6 years (m = 3.2, sd = 2.6) was slightly higher than the mean ADHD score measured at 10 years (m = 2.6, sd = 2.7). The percentage of participants within the ADHD borderline clinical range was 6.8% at 3 years, 5.3% at 6 years, and 6.6% at 10 years. The mean autistic traits score was 4.1 (sd = 4.2) and 1.9% of the participants were within the clinical range of autism.

Table 3. Descriptive statistics of ADHD and autistic traits scores.

	Mean ± SD	Min	Max	clinical cases (N (%)) [a, b]	N
ADHD score at age three years	3.1 ± 2.3	0.0	11.0	42 (6.8%)	618
ADHD score at age six years	3.2 ± 2.6	0.0	12.0	41 (5.3%)	777
ADHD score at age ten years	2.6 ± 2.7	0.0	12.0	39 (6.6%)	588
Autistic traits at age six years	4.1 ± 4.2	0.0	32	12 (1.9%)	622

a. Any ADHD score that falls below the 93rd percentile is considered normal, scores above the 93 percentile are borderline clinical, or clinical cases.[53,54]

b. We utilized the cut-off values recommended by the authors of the SRS for screening in population-based studies (consistent with weighted scores of 1.078 for boys and 1.000 for girls) (Constantino, 2003).

5

Secondary analyses of DAP–ADHD and autistic traits associations

No association between DAP, DE, and DM metabolite concentrations and ADHD traits was observed in any of the 3 urine collection periods during pregnancy (Table 4). Next, similar to the separate regressions, the mutually adjusted DAP and DM metabolite concentrations were not statistically significantly associated with ADHD traits. However, we observed an inverse association between DE metabolite concentrations measured at >25 weeks of gestation in the mutually adjusted model. A 10-fold higher level of DE metabolite concentrations at >25 weeks of gestation was associated with a 0.15 standard deviation lower level of in ADHD traits (95% confidence interval (CI) = −0.28, −0.01).

No association between DAP, DE, and DM metabolite concentrations and autistic traits was observed in any of the 3 urine collection periods during pregnancy (Table 5). Moreover, similar to the separate regressions, the mutually adjusted DAP, DE, and DM metabolite concentrations were not statistically significantly associated with autistic traits.

Sensitivity analyses

No effect modification by sex was observed (P-value for interaction <0.1) with regard to the associations between log10 transformed DAP metabolite concentrations and ADHD traits (Table S4) or autistic traits (Table S5). Further, the results with the use of the clinical cut-off score (Tables S6 and S7), the inverse probability weighted results (Table S8), the results with concentrations below the LOD substituted by LOD/√2 (Table S9), the results with metabolite concentrations expressed as nmol/l with creatinine

Table 4. Difference in standardized ADHD score [a] (and 95% confidence interval) across childhood (and 95% confidence interval) per 10-fold increase in maternal urine dialkyl phosphate metabolite concentrations in nmol/g creatinine, by timing of pregnancy urine sampling and degree of adjustment.

	Childhood ADHD scores (N=781) [b]								
	Unadjusted			Adjusted [c]			Mutually adjusted [d]		
Dialkyl Phosphate Type	B	(95% CI)		B	(95% CI)		B	(95% CI)	
Dialkyl phosphates (total) [e]									
< 18 weeks' gestation	-0.15	-0.33	to 0.04	-0.06	-0.23	to 0.11	-0.07	-0.25	to 0.11
18 – 25 weeks' gestation	-0.03	-0.22	to 0.17	0.05	-0.14	to 0.23	0.08	-0.13	to 0.28
> 25 weeks' gestation	-0.12	-0.31	to 0.07	-0.02	-0.20	to 0.16	-0.03	-0.22	to 0.16
Mean of three urines	-0.20	-0.46	to 0.07	-0.03	-0.28	to 0.23			
Diethyl alkyl phosphates [f]									
< 18 weeks' gestation	-0.09	-0.22	to 0.05	-0.03	-0.15	to 0.10	-0.03	-0.15	to 0.10
18 – 25 weeks' gestation	-0.03	-0.16	to 0.11	0.05	-0.08	to 0.18	0.08	-0.05	to 0.22
> 25 weeks' gestation	-0.23	-0.37	to -0.10	-0.13	-0.26	to 0.00	-0.15	-0.28	to -0.01
Mean of three urines	-0.25	-0.45	to -0.05	-0.08	-0.27	to 0.11			
Dimethyl alkyl phosphates [g]									
< 18 weeks' gestation	-0.11	-0.29	to 0.06	-0.04	-0.20	to 0.12	-0.05	-0.22	to 0.12
18 – 25 weeks' gestation	-0.03	-0.22	to 0.15	0.03	-0.15	to 0.20	0.03	-0.16	to 0.22
> 25 weeks' gestation	-0.05	-0.23	to 0.13	0.03	-0.14	to 0.20	0.02	-0.16	to 0.20
Mean of three urines	-0.14	-0.39	to 0.12	0.00	-0.24	to 0.25			

a. Positive scores indicating more symptomatic behavior.
b. 781 mother-child pairs and 1983 observations.
c. Adjusted for maternal age, maternal psychopathology (0 = no problems, 1= borderline GSI score > 80%), sex of the child, ethnicity (Dutch, other-western and non-western), education (low, intermediate and high), income (low, middle and high), marital status, maternal alcohol consumption (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasionally alcohol consumption during pregnancy and frequently alcohol consumption during pregnancy), non-verbal IQ of the mother, BMI categories (<18.5, 18.5-25, 25-30, 30+), height of the mother, parity categories (0, 1, 2+), and smoking (no smoking during pregnancy, smoked until pregnancy was known, smoked during pregnancy).
d. Adjusted model with the inclusion of the three exposures in one model.
e. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.
f. Diethyl alkyl phosphates is the sum of DEDTP, DETP and DEP.
g. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP and DMP.

concentration added as a separate covariate (Table S10), were all similar to the main analyses. When we adjusted for the measurement error in our exposure biomarkers by assessing averaged urinary DAP concentrations on ADHD and autistic traits with the application of regression calibration, we observed that the effect estimates were stronger (i.e., further away from the null) compared to the results from the primary model, but that the standard errors were increased (Table S11). We observed no difference in the associations when stratified by fruit intake (Tables S12 and S13), and the results with additional adjustment for season of urine collection were similar to the main results (Table S14). Next, no effect modification by SES was observed (Tables S15 and S16). Finally, no associations of child DAP metabolite concentrations with ADHD and autistic traits were detected (Table S17), and these associations did not differ by sex (Table S18).

Table 5. Difference in autistic traits score [a,b] (and 95% confidence interval) per 10-fold increase in maternal urine dialkyl phosphate metabolite concentration in nmol/g creatinine, by when in pregnancy the urine sample was collected.

	Autistics traits score at 6 years (N=622)								
	Unadjusted			Adjusted [c]			Mutually adjusted [d]		
Dialkyl Phosphate Type	B	(95% CI)		B	(95% CI)		B	(95% CI)	
Dialkyl phosphates (total) [e]									
< 18 weeks' gestation	0.05	-0.20	to 0.30	0.15	-0.10	to 0.39	0.15	-0.11	to 0.40
18 – 25 weeks' gestation	-0.07	-0.35	to 0.20	0.02	-0.25	to 0.29	-0.03	-0.32	to 0.26
> 25 weeks' gestation	-0.09	-0.35	to 0.16	0.06	-0.20	to 0.32	0.05	-0.22	to 0.32
Mean of three urines	-0.07	-0.43	to 0.30	0.17	-0.20	to 0.54			
Diethyl alkyl phosphates [f]									
< 18 weeks' gestation	-0.05	-0.22	to 0.12	0.02	-0.15	to 0.18	0.04	-0.13	to 0.21
18 – 25 weeks' gestation	-0.12	-0.30	to 0.06	-0.05	-0.22	to 0.13	-0.02	-0.21	to 0.16
> 25 weeks' gestation	-0.28	-0.48	to -0.07	-0.16	-0.37	to 0.04	-0.16	-0.37	to 0.05
Mean of three urines	-0.29	-0.56	to -0.02	-0.12	-0.39	to 0.15			
Dimethyl alkyl phosphates [g]									
< 18 weeks' gestation	0.10	-0.14	to 0.34	0.17	-0.07	to 0.40	0.16	-0.08	to 0.40
18 – 25 weeks' gestation	-0.04	-0.30	to 0.22	0.05	-0.21	to 0.31	-0.03	-0.30	to 0.25
> 25 weeks' gestation	-0.02	-0.26	to 0.23	0.13	-0.12	to 0.38	0.12	-0.15	to 0.38
Mean of three urines	0.03	-0.32	to 0.39	0.25	-0.11	to 0.61			

a. Square root transformed.
b. Positive scores indicating more symptomatic behavior.
c. Adjusted for maternal age, maternal psychopathology (0 = no problems, 1= borderline GSI score > 80%), sex of the child, ethnicity (Dutch, other-western and non-western), education (low, intermediate and high), income (low, middle and high), marital status, maternal alcohol consumption (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasionally alcohol consumption during pregnancy and frequently alcohol consumption during pregnancy), non-verbal IQ of the mother, BMI categories (<18.5, 18.5-25, 25-30, 30+), height of the mother, parity categories (0, 1, 2+), and smoking (no smoking during pregnancy, smoked until pregnancy was known, smoked during pregnancy).
d. Adjusted model with the inclusion of the three exposures in one model.
e. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.
f. Diethyl alkyl phosphates is the sum of DEDTP, DETP and DEP.
g. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP and DMP.

Discussion

In this large population-based study, higher maternal urinary concentrations of DAP metabolites during pregnancy were not associated with more ADHD traits in 3 to 10- year old children or with autistic traits in 6- year old children. Moreover, no effect modification by sex was observed. Finally, those exposed to higher urinary concentrations of DAP metabolites in childhood also did not have more ADHD traits and autistic traits.

Our results were consistent with a previous study from the Center for Health Assessment of Mothers and Children of Salinas (CHAMACOS) cohort[36] investigating the association between prenatal OP pesticide exposure and ADHD in children aged 2 years, but not

consistent with to 2 other studies, which suggested that prenatal OP pesticide exposure was associated with ADHD.[37,41] Marks et al. (2010), using data from the same birth cohort study as Eskenazi et al. (2007), found prenatal OP pesticide exposure to be associated with more ADHD traits at child age 5 years. Rauh et al. (2006), using data from a study population from inner-city New York, found that prenatal exposure to chlorpyrifos measured in blood was associated with offspring's ADHD traits at the age of 3 years. Our null results for autistic traits are not in line with the results in children aged 14 years from a previous study of the CHAMACOS cohort,[42] but consistent with findings of the Mount Sinai Children's Environmental Health Study[45] and Health Outcomes and Measures of the Environment (HOME) Study[43] in children aged 8 years that also measured autistic traits with the use of the SRS in children.

Several other researchers found some evidence for an association between prenatal OP pesticide exposure and autism in studies of clinical cases[73,83] or PDD cases aged 2 to 3 years.[36,37] However, often the associations were limited to subgroups.[41,44,45] Marks et al. (2010) reported an association between OP and ADHD traits, only in boys, Furlong et al. (2014) between prenatal DE metabolite concentrations and autistic traits (SRS-score), only in boys and in black children (aged 5 years), and Philippat et al. (2018), between DMTP metabolites and ASD in girls (aged 3 years) using data from the Markers of Autism Risk in Babies–Learning Early Signs (MARBLES) mother-child cohort. We did not replicate these findings.

Next, we explored whether children with higher DAP metabolite concentrations at age 6 years had higher levels of ADHD or autistic traits since few studies using data from NHANES and Children Pesticide Survey (CPS) reported these associations[81,82] in children aged 7 to 15 years. We found no evidence for any association.

The inconsistency of results may be related to differences in OP pesticide exposure sources across study areas, the exposure mixture, or the exposure assessment methodology. Most previous studies were carried out in the US and the exposure mixture of OP pesticides in Europe is different than in the US due to differences in regulations regarding the use of OP pesticides. Within the US, the majority of the studies were conducted in California[36,41,42,44,73,83] where agricultural insecticides are extensively used. In contrast, the Generation R population lives in urban settings, where the main route of exposure is through the ingestion of food, and most likely fruits.[5] Furthermore, DAP metabolites are non-specific biomarkers of OP pesticide exposure, thus it is possible that the mixture of parent compounds differed across cohorts and thus toxicity varies even if DAP levels were similar. Also, because preformed DAP metabolites are present on food crops and in the natural environment,[7-9] it is uncertain which amount of the total DAP metabolite concentrations is due to the ingestion of the less toxic DAP metabolites from the natural

environment.[10] Finally, the majority of these studies, including our study, measured DAP metabolites in urine as a biomarker of OP pesticide exposure. However, some studies took a different approach. For example, 3 studies used maternal residence near agricultural pesticide applications to estimate exposure,[42,73,83] and another study used parent compounds measured in umbilical cord blood collected at delivery to measure the level of OP pesticide exposure.[37] Differences in exposure sources, mixtures, routes, and assessments across studies complicate the comparison.

Discrepancies in the results of studies may be related to differences in study populations. For example, the majority of our study sample consisted of Dutch participants with a relatively high SES. Apart from the study of Millenson et al. (2017), that reported similar results, most previous studies mainly included participants from ethnic minorities or with low SES.[36,37,41,42,45] It is conceivable that these populations with low SES were exposed to unobserved background risk factors that are related to the likelihood of OP pesticide exposure and the risk for ADHD or autistic traits. Although these studies adjusted for SES related confounders, there may still be residual confounding. In our study, the unadjusted models predicting ADHD and autistic traits were almost all in a negative direction, suggesting a protective effect. After adjustment (including SES related confounders), the observed inverse associations were closer to the null or in the positive direction (albeit clearly non-statistically significant), suggesting the presence of some observed negative confounding.[84] However, the inverse association for DE metabolite concentrations measured at >25 weeks of gestation and ADHD traits in the mutually adjusted model remained. We must be cautious in interpreting this association which may be a result of multiple testing. Also, this finding is contrary to the findings of Eskenazi et al. (2007) and Marks et al. (2010) who observed that higher DE metabolite concentrations were associated with more PDD and ADHD problems. Although we included many potential confounders in the model, we cannot rule out that other underlying factors such as a healthier lifestyle among those exposed to OP pesticides, might have resulted in unobserved negative confounding. Millenson et al. (2017) and Cartier et al. (2016) observed a similar pattern of negative confounding in the association between maternal DE metabolite concentrations and child neurodevelopment among persons with relatively high SES. These studies showed a significant unadjusted association of maternal DE metabolite concentrations with autistic traits and verbal comprehensive score in an unexpected direction (i.e. higher DE metabolite concentrations–less problems). However, after adjustment the observed inverse associations were closer to the null.

Another notable difference between our study and other studies investigating the association between OP pesticide exposure and ADHD and autistic traits is that in this study the DAP metabolite concentrations (308–316 nmol/g creatinine) were 2–3 times higher than in previous studies.[2,4,36,44,85-87] This could be due to higher consumption of fruits and

vegetables, higher SES (which is associated with more healthy food consumption), or due to the extensive farming practices in the Netherlands.[5,88] Nevertheless, we did not find any evidence for an association between OP pesticide exposure and ADHD and autistic traits.

A few limitations of the present study need to be considered. As mentioned above, DAP metabolites also exist in the food supply.[7-9] It is therefore uncertain to what extent the total DAP metabolite concentrations are due to the OP pesticide exposure or due to the ingestion of the less toxic DAP metabolites.[10] Further, DAP metabolite concentrations provide non-specific information about the cumulative exposure to a class of OP pesticides rather than a single OP pesticide.[89] It is therefore unknown to which specific OP parent pesticide(s) our study population was exposed. However, the estimation of urinary DAP metabolite concentrations is considered an appropriate and useful tool to identify and compare levels of OP pesticide exposure among various population.[11]

Urinary DAP metabolites have a short half-life. These metabolites are excreted in urine within approximately 24 h, which implies that the measured DAP concentrations may vary from day-today within each subject[46] giving rise to chance findings. It would be ideal to collect a broad range of urine specimens more often during pregnancy,[90] especially since the ICCs of DAP metabolite concentrations are modest.[70] To achieve excellent reliability of OP pesticide exposure over pregnancy, 4 weekly pools of 15 to 20 urine samples would be needed.[91] The present study includes 3 spot-urine measures of maternal DAP metabolite concentrations per subject from a large sample. Although our sampling frequency is higher than that of most other studies of maternal urinary DAP metabolite concentrations and neurodevelopment,[32] the urinary measurement variability may have still resulted in attenuated estimates due to measurement error in our biomarkers.[90] Indeed, adjusting for measurement error with regression calibration resulted in effect estimates that were further from the null albeit more imprecise.[92]

Another limitation of this study is the absence of information about the exact time of spot urine sampling. Because the urine spot samples were collected between 8 am and 8 pm, there may have been a combination of first morning and random spot samples. Concentrations of chemicals, urine volume and the rate of excretion vary with fluid intake, time of day, and other factors.[93-95] Although time of sample collection is unlikely to confound the association between DAP metabolite concentrations and child neurodevelopmental outcomes, the use of DAP metabolite concentrations without the adjustment of timing of urine sampling may have resulted in high intra-individual variability and less precise associations. To increase precision, we adjusted the main analyses for season of urine collection. The results were essentially the same.

Although the percentage of participants above the SRS cut-off value for screening in the population (1.9%)[59] was similar to the overall prevalence of ASD (0.6–2.2%) in children aged 8 years,[96] the percentages of participants considered borderline clinical or clinical ADHD cases[53,54] in our study (5.3–6.8%) was lower than the prevalence of ADHD (7–14%).[97] Because the children in our study have parents with a higher education, more income, and who were less likely to smoke during pregnancy, it is possible that the children were generally healthier than the source population. This could have resulted in lower variability in ADHD and autistic traits which may explain our null findings. Also, the differences in characteristics between our study sample and the source population, i.e., the total Generation R cohort, may have introduced selection bias. Yet, we used inverse probability weighting to account for potential selection bias and the results were essentially the same.

A strength of this study is the evaluation of ADHD and ASD phenotype on a continuous spectrum rather than the use of a clinical cut-off (presence versus absence of disease). For example, several other studies used a clinical diagnosis of ASD[44,73,83] or clinical cut-off.[36,37] Other studies, including ours, relied on the CBCL and SRS measured on a continuous scale. Although the use of the SRS and CBCL does not provide a clinical diagnosis, it has many advantages. For instance, the use of a continuous scale increases power and allow us to account for children with fewer symptoms who may not have met the diagnostic criteria when a clinical cut-off is used, and reduces the impact of outcome misclassification.[98] Further, the use of repeated measures of ADHD traits is another strength. Repeated measures of ADHD traits increase statistical power by allowing for missing observation at various time points, and gave us the ability to include a random slope for time to account for varying effects of time on ADHD across subjects.[99-103] Finally, the availability of a broad range of contextual information for confounder adjustment is another strength of this study.

In conclusion, in this study of maternal urinary DAP metabolite concentrations during pregnancy, we did not observe associations with ADHD and autistic traits in children. These are important null observations because of the relatively high background DAP concentrations across pregnancy, the relatively large sample size, and the 10-year follow-up of the offspring. Given the measurement error inherent in our OP pesticide exposure biomarkers, future studies using more urine samples are needed to accurately measure OP pesticide exposure over pregnancy in relation to ADHD and autistic traits. Further, the Generation R Study is representative of an urban population with varying ethnicities, SES, and educational level, and therefore less generalizable to populations where the OP pesticide exposure sources may differ.

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Supplemental Material

Table S1. Variables used in logistic regression model to calculate inverse probability.

Variables	Explored	Included
Maternal educational level	x	x
Maternal ethnicity	x	x
Maternal age	x	
Maternal parity	x	x
Maternal alcohol use during pregnancy	x	x
Maternal tobacco use during pregnancy	x	
Maternal body mass index	x	x
Household income during pregnancy	x	
Marital status during pregnancy	x	
Maternal IQ	x	
Maternal psychopathology	x	
Breastfeeding	x	
Child's sex	x	
Child's birth weight	x	
Gestational age at birth	x	x
Child IT-HOME score	x	x

Table S2. Intraclass correlation coefficients for log10 transformed maternal urine dialkyl phosphate metabolite concentrations in nmol/g creatinine (n=781).

	ICC [a]	ICC [b]
Dialkyl phosphates (total) [c]	0.259	0.511
Diethyl alkyl phosphates [d]	0.217	0.454
Dimethyl alkyl phosphates [e]	0.230	0.473

a. ICC = Intraclass Correlation Coefficients calculated using a single-measurement, absolute-agreement, and 2-way mixed-effects model.
b. ICC = Intraclass Correlation Coefficients calculated using a mean of three measurements, absolute-agreement, and 2-way mixed-effects model.
c. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.
d. Diethyl alkyl phosphates is the sum of DEDTP, DETP and DEP.
e. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP and DMP.

Table S3. Correlation coefficients [a] for log10 transformed maternal and 6-year-old child urine dialkyl phosphate metabolite concentrations in nmol/g creatinine.

Pearson correlation coefficients	< 18 weeks	18 – 25 weeks	> 25 weeks	Child at age 6
Dialkyl phosphates (total) [b]				
< 18 weeks of gestation	1			
18 – 25 weeks of gestation	0.26**	1		
> 25 weeks of gestation	0.18**	0.35**	1	
child at age 6	0.01	0.02	0.01	1
Diethyl alkyl phosphates [c]				
< 18 weeks of gestation	1			
18 – 25 weeks of gestation	0.22**	1		
> 25 weeks of gestation	0.14**	0.25**	1	
child at age 6	0.02	-0.01	0.05	1
Dimethyl alkyl phosphates [d]				
< 18 weeks of gestation	1			
18 – 25 weeks of gestation	0.23**	1		
> 25 weeks of gestation	0.15**	0.33**	1	
child at age 6	-0.01	0.02	0.01	1

a. N=781 between correlations of maternal DAP urinary concentrations across pregnancy, N=747 between correlations of maternal and child DAP urinary concentrations.
b. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.
c. Diethyl alkyl phosphates is the sum of DEDTP, DETP and DEP.
d. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP and DMP.
** P<0.01.

Table S4. Sex stratified difference in standardized ADHD score (and 95% confidence interval) across childhood per 10-fold increase in maternal urine dialkyl phosphate metabolite concentration in nmol/g creatinine.

	Childhood ADHD scores (N=781) [a]							
	Boys			Girls				
Total dialkyl phosphates [b]	B	95%CI		B	95%CI		P-value [c]	P-value [d]
None								
< 18 weeks of gestation	-0.19	-0.45	to 0.07	-0.14	-0.38	to 0.10	0.774	0.774
18 – 25 weeks of gestation	0.07	-0.21	to 0.34	-0.20	-0.47	to 0.06	0.173	0.173
> 25 weeks of gestation	-0.19	-0.47	to 0.09	-0.07	-0.31	to 0.17	0.523	0.523
Mean of three urines	-0.21	-0.59	to 0.18	-0.27	-0.62	to 0.08	0.827	0.827
Adjusted [e]								
< 18 weeks of gestation	-0.10	-0.35	to 0.15	-0.01	-0.25	to 0.23	0.652	0.608
18 – 25 weeks of gestation	0.06	-0.21	to 0.32	-0.06	-0.33	to 0.20	0.565	0.560
> 25 weeks of gestation	-0.09	-0.36	to 0.18	0.08	-0.16	to 0.33	0.337	0.351
Mean of three urines	-0.10	-0.47	to 0.28	0.01	-0.35	to 0.37	0.692	0.670
Mutually adjusted [f]								
< 18 weeks of gestation	-0.12	-0.39	to 0.15	-0.01	-0.26	to 0.23	0.614	0.599
18 – 25 weeks of gestation	0.12	-0.17	to 0.40	-0.10	-0.39	to 0.18	0.547	0.538
> 25 weeks of gestation	-0.10	-0.38	to 0.18	0.12	-0.15	to 0.38	0.358	0.354

a. Positive scores indicating more symptomatic behavior.
b. Total Dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.
c. P-value for interaction.
d. P-value for interaction based on the augmented product term approach (Buckley et al. 2017).
e. Adjusted for maternal age, maternal psychopathology (0 = no problems, 1= borderline:: GSI score > 80%), ethnicity (Dutch, other-Western and non-Western), education (low, intermediate and high), income (low, middle and high), marital status, maternal alcohol consumption (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasionally alcohol consumption during pregnancy and frequently alcohol consumption during pregnancy), non-verbal IQ of the mother, BMI categories (<18.5, 18.5-25, 25-30, 30+), height (≤168,>168) of the mother, parity categories (0, 1, 2+), and smoking (no smoking during pregnancy, smoked until pregnancy was known, smoked during pregnancy).
f. adjusted model with the inclusion of the three exposures in one model.

Table S5. Sex stratified difference in autistic traits score (and 95% confidence interval) per 10-fold increase in maternal urine dialkyl phosphate metabolite concentration in nmol/g creatinine.

	Autistics traits score at 6 years (N=622) [a,b]									
	Boys				Girls					
Total dialkyl phosphates [c]	B	95%CI			B	95%CI			P-value [d]	P-value [e]
None										
< 18 weeks of gestation	0.04	-0.30	to	0.38	0.02	-0.34	to	0.38	0.923	0.923
18 – 25 weeks of gestation	-0.08	-0.44	to	0.28	-0.09	-0.50	to	0.31	0.963	0.963
> 25 weeks of gestation	-0.16	-0.52	to	0.20	-0.03	-0.39	to	0.34	0.601	0.601
Mean of three urines	-0.12	-0.61	to	0.37	-0.06	-0.61	to	0.48	0.872	0.872
Adjusted [f]										
< 18 weeks of gestation	0.14	-0.21	to	0.48	0.13	-0.23	to	0.50	0.887	0.989
18 – 25 weeks of gestation	-0.07	-0.43	to	0.30	0.05	-0.37	to	0.47	0.654	0.677
> 25 weeks of gestation	-0.02	-0.39	to	0.35	0.08	-0.30	to	0.46	0.631	0.714
Mean of three urines	0.00	-0.39	to	0.38	0.12	-0.25	to	0.49	0.585	0.699
Mutually adjusted [g]										
< 18 weeks of gestation	0.17	-0.19	to	0.53	0.12	-0.25	to	0.49	0.908	0.954
18 – 25 weeks of gestation	-0.12	-0.52	to	0.28	0.01	-0.44	to	0.46	0.579	0.615
> 25 weeks of gestation	0.00	-0.39	to	0.38	0.06	-0.35	to	0.46	0.620	0.722

a. Square root transformed.
b. Positive scores indicating more symptomatic behavior.
c. Total Dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.
d. P-value for interaction.
e. P-value for interaction based on the augmented product term approach (Buckley et al. 2017).
f. Adjusted for maternal age, maternal psychopathology (0 = no problems, 1= borderline: GSI score > 80%), ethnicity (Dutch, other-Western and non-Western), education (low, intermediate and high), income (low, middle and high), marital status, maternal alcohol consumption (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasionally alcohol consumption during pregnancy and frequently alcohol consumption during pregnancy), non-verbal IQ of the mother, BMI categories (<18.5, 18.5-25, 25-30, 30+), height (≤168,>168) of the mother, parity categories (0, 1, 2+), and smoking (no smoking during pregnancy, smoked until pregnancy was known, smoked during pregnancy).
g. adjusted model with the inclusion of the three exposures in one model.

Table S6. Odds ratios (OR) (and 95% confidence intervals) for the associations between log10 transformed maternal urine dialkyl phosphate metabolite concentrations in nmol/g creatinine and borderline clinical range of ADHD across childhood.

	Clinical cases (n=39-42)				Cases with ADHD score >80th percentile			
	Unadjusted		Adjusted [a]		Unadjusted		Adjusted [a]	
Dialkyl Phosphate Type	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Dialkyl (total) phosphates [b]								
< 18 weeks of gestation	0.6	0.2 to 1.8	0.7	0.3 to 1.8	0.7	0.4 to 1.1	0.8	0.5 to 1.3
18 – 25 weeks of gestation	1.4	0.5 to 4.1	1.5	0.6 to 3.8	1.1	0.7 to 1.9	1.2	0.7 to 2.1
> 25 weeks of gestation	0.6	0.2 to 1.6	0.7	0.3 to 1.8	0.8	0.5 to 1.3	1.0	0.6 to 1.7
Mean of three urines	0.7	0.2 to 2.7	0.7	0.2 to 2.7	0.7	0.3 to 1.4	0.7	0.3 to 1.4
Diethyl alkyl phosphates [c]								
< 18 weeks of gestation	0.8	0.4 to 1.7	0.9	0.5 to 1.8	0.8	0.6 to 1.2	0.9	0.6 to 1.4
18 – 25 weeks of gestation	1.1	0.5 to 2.5	1.2	0.6 to 2.5	1.0	0.7 to 1.5	1.1	0.7 to 1.6
> 25 weeks of gestation	0.5	0.3 to 0.9	0.6	0.3 to 1.0	0.5	0.4 to 0.8	0.6	0.4 to 0.9
Mean of three urines	0.5	0.2 to 1.5	0.5	0.2 to 1.5	0.5	0.3 to 0.9	0.5	0.3 to 0.9
Dimethyl alkyl phosphates [d]								
< 18 weeks of gestation	0.7	0.3 to 1.9	0.8	0.3 to 1.8	0.7	0.5 to 1.2	0.8	0.5 to 1.3
18 – 25 weeks of gestation	1.3	0.5 to 3.8	1.4	0.6 to 3.4	1.1	0.6 to 1.7	1.2	0.7 to 1.9
> 25 weeks of gestation	0.7	0.3 to 1.9	0.9	0.4 to 2.1	1.0	0.6 to 1.5	1.2	0.7 to 1.9
Mean of three urines	0.8	0.2 to 3.1	1.0	0.3 to 3.3	0.8	0.4 to 1.6	1.1	0.5 to 2.1

a. Because of the reduced power due to dichotomization of the outcome, the models were adjusted for the minimal sufficient adjustment set of variables (with the inclusion of two important ancestors of the outcome) given by the Dagitty software (Textor et al. 2017) for estimating the total effect of DAP metabolite concentrations on ADHD traits: maternal age, maternal BMI, maternal fruit intake, maternal education (proxy of socio-economic status), and sex of the child.

b. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.

c. Diethyl alkyl phosphates is the sum of DEDTP, DETP and DEP.

d. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP and DMP.

Table S7. Odds ratios (OR) (and 95% confidence intervals) for the associations between log10 transformed maternal urine dialkyl phosphate metabolite concentrations in nmol/g creatinine and clinical range of autism at child age 6 years.

	Clinical cases (N=12)				Cases with autistic traits score >80th percentile			
	Unadjusted		Adjusted [a]		Unadjusted		Adjusted [a]	
Dialkyl Phosphate Type	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Dialkyl (total) phosphates [b]								
< 18 weeks of gestation	3.3	0.6 to 19.4	3.9	0.7 to 21.7	1.0	0.6 to 1.9	1.2	0.6 to 2.1
18 – 25 weeks of gestation	1.9	0.3 to 13.6	3.1	0.4 to 24.7	0.8	0.4 to 1.5	0.8	0.4 to 1.6
> 25 weeks of gestation	0.5	0.1 to 3.3	0.7	0.1 to 4.7	0.6	0.3 to 1.1	0.7	0.4 to 1.4
Mean of three urines	2.4	0.2 to 34.5	5.0	0.3 to 82.1	0.6	0.3 to 1.5	0.8	0.3 to 2.1
Diethyl alkyl phosphates [c]								
< 18 weeks of gestation	0.8	0.3 to 2.3	0.9	0.3 to 2.8	0.9	0.6 to 1.4	1.0	0.7 to 1.5
18 – 25 weeks of gestation	0.9	0.3 to 2.7	1.1	0.3 to 4.3	0.7	0.5 to 1.1	0.7	0.5 to 1.1
> 25 weeks of gestation	0.6	0.1 to 2.7	0.8	0.2 to 3.3	0.6	0.4 to 1.0	0.7	0.4 to 1.2
Mean of three urines	0.6	0.1 to 3.5	0.8	0.1 to 5.7	0.5	0.3 to 1.0	0.6	0.3 to 1.2
Dimethyl alkyl phosphates [d]								
< 18 weeks of gestation	4.4	0.8 to 23.9	5.0	1.0 to 26.4	1.0	0.6 to 1.9	1.2	0.6 to 2.1
18 – 25 weeks of gestation	2.3	0.3 to 15.0	3.6	0.5 to 26.4	0.8	0.4 to 1.5	0.9	0.5 to 1.7
> 25 weeks of gestation	0.5	0.1 to 2.9	0.7	0.1 to 4.1	0.7	0.4 to 1.2	0.8	0.4 to 1.5
Mean of three urines	3.2	0.2 to 45.2	6.6	0.4 to 104.6	0.7	0.3 to 1.6	0.9	0.4 to 2.2

a. Because of the reduced power due to dichotomization of the outcome, the models were adjusted for the minimal sufficient adjustment set of variables (with the inclusion of two important ancestors of the outcome) given by the Dagitty software (Textor et al. 2017) for estimating the total effect of DAP metabolite concentrations on ADHD traits: maternal age, maternal BMI, maternal fruit intake, maternal education (proxy of socio-economic status), and sex of the child.

b. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.

c. Diethyl alkyl phosphates is the sum of DEDTP, DETP and DEP.

d. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP and DMP.

Table S8. Inverse probability weighted [a] associations (and 95% confidence intervals) between log10 transformed maternal urine dialkyl phosphate metabolite concentrations in nmol/g creatinine and standardized ADHD scores across childhood and autistic traits scores at age 6 years.

	ADHD scores across childhood (N=781)		Autistic traits scores at age 6 years [b] (N=622)	
Dialkyl phosphates (total) [c]	B	95% CI	B	95% CI
Unadjusted				
< 18 weeks of gestation	-0.15	-0.33 to 0.03	0.05	-0.20 to 0.30
18 – 25 weeks of gestation	-0.04	-0.23 to 0.15	-0.06	-0.33 to 0.21
> 25 weeks of gestation	-0.12	-0.31 to 0.07	-0.09	-0.35 to 0.17
Mean of three urines	-0.21	-0.47 to 0.06	-0.06	-0.42 to 0.31
Adjusted [d]				
< 18 weeks of gestation	-0.06	-0.23 to 0.11	0.14	-0.11 to 0.38
18 – 25 weeks of gestation	0.04	-0.14 to 0.23	0.03	-0.24 to 0.30
> 25 weeks of gestation	-0.02	-0.20 to 0.16	0.05	-0.21 to 0.30
Mean of three urines	-0.03	-0.29 to 0.22	0.16	-0.21 to 0.52
Mutually adjusted [e]				
< 18 weeks of gestation	-0.07	-0.25 to 0.11	0.14	-0.11 to 0.39
18 – 25 weeks of gestation	0.07	-0.13 to 0.27	-0.02	-0.31 to 0.28
> 25 weeks of gestation	-0.03	-0.22 to 0.16	0.03	-0.24 to 0.30

a. Weighted for the inverse probability to be included in the study sample.
b. square root transformed.
c. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.
d. Adjusted for maternal age, maternal psychopathology (0 = no problems, 1= borderline: GSI score > 80%), sex of the child, ethnicity (Dutch, other-Western and non-Western), education (low, intermediate and high), income (low, middle and high), marital status, maternal alcohol consumption (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasionally alcohol consumption during pregnancy and frequently alcohol consumption during pregnancy), non-verbal IQ of the mother, BMI categories (<18.5, 18.5-25, 25-30, 30+), height (≤168,>168) of the mother, parity categories (0, 1, 2+), and smoking (no smoking during pregnancy, smoked until pregnancy was known, smoked during pregnancy).
e. Adjusted model with the inclusion of the three exposures in one model.

Table S9. Associations (and 95% confidence intervals) between log10 transformed maternal urine dialkyl phosphate metabolite concentrations in nmol/g creatinine and standardized ADHD scores across childhood and autistic traits scores at age 6 years, in which the values below the LOD are replaced with LOD/√2.

	ADHD scores across childhood (N=781)			Autistic traits scores at age 6 years [a] N=623)		
Dialkyl phosphates (total) [b]	B	95% CI		B	95% CI	
Unadjusted						
< 18 weeks of gestation	-0.16	-0.34	to 0.03	0.05	-0.20	to 0.30
18 – 25 weeks of gestation	-0.04	-0.23	to 0.15	-0.08	-0.35	to 0.19
> 25 weeks of gestation	-0.12	-0.31	to 0.06	-0.09	-0.35	to 0.17
Mean of three urines	-0.22	-0.48	to 0.05	-0.07	-0.44	to 0.30
Adjusted [c]						
< 18 weeks of gestation	-0.06	-0.23	to 0.11	0.15	-0.10	to 0.39
18 – 25 weeks of gestation	0.04	-0.14	to 0.23	0.02	-0.26	to 0.29
> 25 weeks of gestation	-0.02	-0.20	to 0.16	0.07	-0.19	to 0.33
Mean of three urines	-0.03	-0.29	to 0.22	0.17	-0.20	to 0.54
Mutually adjusted [d]						
< 18 weeks of gestation	-0.07	-0.25	to 0.10	0.15	-0.10	to 0.39
18 – 25 weeks of gestation	0.07	-0.13	to 0.27	0.02	-0.26	to 0.29
> 25 weeks of gestation	-0.03	-0.22	to 0.16	0.07	-0.19	to 0.33

a. square root transformed.

b. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.

c. Adjusted for maternal age, maternal psychopathology (0 = no problems, 1= borderline: GSI score > 80%), sex of the child, ethnicity (Dutch, other-Western and non-Western), education (low, intermediate and high), income (low, middle and high), marital status, maternal alcohol consumption (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasionally alcohol consumption during pregnancy and frequently alcohol consumption during pregnancy), non-verbal IQ of the mother, BMI categories (<18.5, 18.5-25, 25-30, 30+), height (≤168,>168) of the mother, parity categories (0, 1, 2+), and smoking (no smoking during pregnancy, smoked until pregnancy was known, smoked during pregnancy).

d. Adjusted model with the inclusion of the three exposures in one model.

Table S10. Associations (and 95% confidence intervals) between log10 transformed maternal urine dialkyl phosphate metabolite concentrations in nmol/l and standardized ADHD scores across childhood and autistic traits scores at age 6 years.

	ADHD scores across childhood (N=781)			Autistic traits scores at age 6 years (N=622)		
Dialkyl phosphates (total) [b]	B	95% CI		B	95% CI	
Unadjusted						
< 18 weeks of gestation	-0.11	-0.28 to	0.06	0.06	-0.18 to	0.29
18 – 25 weeks of gestation	0.00	-0.17 to	0.18	0.05	-0.20 to	0.29
> 25 weeks of gestation	-0.11	-0.29 to	0.07	-0.12	-0.37 to	0.12
Mean of three urines	-0.13	-0.39 to	0.12	0.07	-0.26 to	0.41
Adjusted [c]						
< 18 weeks of gestation	-0.05	-0.21 to	0.11	0.09	-0.14 to	0.32
18 – 25 weeks of gestation	0.08	-0.09 to	0.24	0.12	-0.12 to	0.36
> 25 weeks of gestation	-0.03	-0.20 to	0.14	-0.01	-0.25 to	0.23
Mean of three urines	-0.01	-0.25 to	0.23	0.15	-0.18 to	0.48
Mutually adjusted [d]						
< 18 weeks of gestation	-0.06	-0.22 to	0.11	0.09	-0.14 to	0.33
18 – 25 weeks of gestation	0.10	-0.08 to	0.27	0.11	-0.15 to	0.37
> 25 weeks of gestation	-0.05	-0.22 to	0.13	-0.05	-0.30 to	0.20

a. square root transformed.

b. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.

c. Adjusted for creatinine, maternal age, maternal psychopathology (0 = no problems, 1= borderline: GSI score > 80%), sex of the child, ethnicity (Dutch, other-Western and non-Western), education (low, intermediate and high), income (low, middle and high), marital status, maternal alcohol consumption (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasionally alcohol consumption during pregnancy and frequently alcohol consumption during pregnancy), non-verbal IQ of the mother, BMI categories (<18.5, 18.5-25, 25-30, 30+), height (≤168,>168) of the mother, parity categories (0, 1, 2+), and smoking (no smoking during pregnancy, smoked until pregnancy was known, smoked during pregnancy).

d. Adjusted model with the inclusion of the three exposures in one model.

Table S11. Regression calibration estimated associations [a] (and 95% confidence intervals) between log10 transformed maternal urine dialkyl phosphate metabolite concentrations in nmol/g creatinine and standardized ADHD scores across childhood and autistic traits scores at age 6 years.

	Naive model		Regression calibration model	
ADHD scores across childhood (N=781)	B	95%CI	B	95%CI
Averaged dialkyl phosphates (total) [b]	-0.03	-0.28 to 0.23	-0.09	-1.00 to 0.82
Averaged diethyl alkyl phosphates [c]	-0.08	-0.27 to 0.11	-0.42	-1.26 to 0.43
Averaged dimethyl alkyl phosphates [d]	0.00	-0.24 to 0.25	0.03	-1.08 to 1.15
	Naive model		Regression calibration model	
Autistic traits scores (N=622) [e]	B	95%CI	B	95%CI
Averaged dialkyl phosphates (total) [b]	0.17	-0.20 to 0.54	0.54	-0.87 to 1.95
Averaged diethyl alkyl phosphates [c]	-0.12	-0.39 to 0.15	-0.52	-1.77 to 0.73
Averaged dimethyl alkyl phosphates [d]	0.25	-0.11 to 0.61	1.06	-0.71 to 2.83

a. Adjusted for maternal age, maternal psychopathology (0 = no problems, 1= borderline: GSI score > 80%), sex of the child, ethnicity (Dutch, other-Western and non-Western), education (low, intermediate and high), income (low, middle and high), marital status, maternal alcohol consumption (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasionally alcohol consumption during pregnancy and frequently alcohol consumption during pregnancy), non-verbal IQ of the mother, BMI categories (<18.5, 18.5-25, 25-30, 30+), height (≤168,>168) of the mother, parity categories (0, 1, 2+), and smoking (no smoking during pregnancy, smoked until pregnancy was known, smoked during pregnancy).

b. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.

c. Diethyl alkyl phosphates is the sum of DEDTP, DETP and DEP.

d. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP and DMP.

e. square root transformed.

Table S12. Fruit intake [a] stratified difference in standardized ADHD score (and 95% confidence interval) across childhood per 10-fold increase in maternal urine dialkyl phosphate metabolite concentrations in nmol/g creatinine.

	ADHD score (N=781)								
	<75 grams fruit a day (N=156)				≥75 grams fruit a day (N=625)				
Total dialkyl phosphates [b]	B	95%CI			B	95%CI			P interaction
None									
< 18 weeks of gestation	-0.10	-0.54	to	0.34	-0.12	-0.33	to	0.09	0.912
18 – 25 weeks of gestation	0.02	-0.51	to	0.54	0.01	-0.23	to	0.24	0.969
> 25 weeks of gestation	-0.25	-0.72	to	0.21	-0.04	-0.25	to	0.17	0.388
Mean of three urines	-0.22	-0.89	to	0.46	-0.11	-0.42	to	0.21	0.787
Adjusted [c]									
< 18 weeks of gestation	-0.16	-0.61	to	0.30	-0.05	-0.26	to	0.16	0.928
18 – 25 weeks of gestation	-0.03	-0.52	to	0.46	0.08	-0.14	to	0.31	0.790
> 25 weeks of gestation	-0.22	-0.71	to	0.28	0.09	-0.12	to	0.30	0.498
Mean of three urines	-0.27	-0.93	to	0.39	0.08	-0.23	to	0.40	0.812
Mutually adjusted [d]									
< 18 weeks of gestation	-0.14	-0.62	to	0.35	-0.07	-0.28	to	0.14	0.923
18 – 25 weeks of gestation	0.08	-0.47	to	0.63	0.08	-0.15	to	0.31	0.775
> 25 weeks of gestation	-0.22	-0.77	to	0.34	0.08	-0.14	to	0.30	0.515

a. Fruit intake stratified for <75 grams (<1st quantile) versus ≥75 (≥1st quantile)

b. Total Dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.

c. Adjusted for maternal age, maternal psychopathology (0 = no problems, 1= borderline: GSI score > 80%), sex of the child, ethnicity (Dutch, other-Western and non-Western), education (low, intermediate and high), income (low, middle and high), marital status, maternal alcohol consumption (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasionally alcohol consumption during pregnancy and frequently alcohol consumption during pregnancy), non-verbal IQ of the mother, BMI categories (<18.5, 18.5-25, 25-30, 30+), height (≤168,>168) of the mother, parity categories (0, 1, 2+), and smoking (no smoking during pregnancy, smoked until pregnancy was known, smoked during pregnancy).

d. adjusted model with the inclusion of the three exposures in one model.

Table S13. Fruit intake [a] stratified difference in autistic traits score (and 95% confidence interval) at age 6 years per 10-fold increase in maternal urine dialkyl phosphate metabolite concentrations in nmol/g creatinine.

	Child autistic traits score (N=622) [b]						
	<75 grams fruit a day (N=109)			≥75 grams fruit a day (N=513)			
Total dialkyl phosphates [c]	B	95%CI		B	95%CI		P interaction
None							
< 18 weeks of gestation	0.11	-0.49	to 0.70	0.10	-0.19	to 0.38	0.981
18 – 25 weeks of gestation	0.12	-0.58	to 0.81	-0.08	-0.40	to 0.23	0.605
> 25 weeks of gestation	0.11	-0.53	to 0.76	-0.11	-0.41	to 0.20	0.546
Mean of three urines	0.21	-0.68	to 1.10	-0.06	-0.50	to 0.38	0.602
Adjusted [d]							
< 18 weeks of gestation	0.00	-0.63	to 0.64	0.15	-0.13	to 0.44	0.689
18 – 25 weeks of gestation	0.01	-0.78	to 0.80	-0.01	-0.32	to 0.30	0.422
> 25 weeks of gestation	0.25	-0.45	to 0.95	0.04	-0.27	to 0.35	0.447
Mean of three urines	0.17	-0.82	to 1.15	0.15	-0.30	to 0.59	0.632
Mutually adjusted [e]							
< 18 weeks of gestation	-0.03	-0.72	to 0.66	0.16	-0.13	to 0.44	0.680
18 – 25 weeks of gestation	-0.08	-0.94	to 0.79	-0.05	-0.38	to 0.28	0.460
> 25 weeks of gestation	0.28	-0.46	to 1.02	0.04	-0.28	to 0.36	0.460

a. Fruit intake stratified for <75 grams (<1st quantile) versus ≥75 (≥1st quantile).
b. Square root transformed.
c. Total Dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.
d. Adjusted for maternal age, maternal psychopathology (0 = no problems, 1= borderline: GSI score > 80%), sex of the child, ethnicity (Dutch, other-Western and non-Western), education (low, intermediate and high), income (low, middle and high), marital status, maternal alcohol consumption (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasionally alcohol consumption during pregnancy and frequently alcohol consumption during pregnancy), non-verbal IQ of the mother, BMI categories (<18.5, 18.5-25, 25-30, 30+), height (≤168,>168) of the mother, parity categories (0, 1, 2+), and smoking (no smoking during pregnancy, smoked until pregnancy was known, smoked during pregnancy).
e. adjusted model with the inclusion of the three exposures in one model.

Table S14. Associations (and 95% confidence intervals) between log10 transformed maternal urine dialkyl phosphate metabolite concentrations in nmol/g creatinine and standardized ADHD scores across childhood and autistic traits scores at age 6 years, with additional adjustment for the season of urine collection.

	ADHD scores across childhood (N=781)			Autistic traits scores at age 6 years (N=622)		
Dialkyl phosphates (total) [b]	B	95% CI		B	95% CI	
Unadjusted [c]						
< 18 weeks of gestation	-0.15	-0.33	0.03	0.05	-0.20	0.30
18 – 25 weeks of gestation	-0.04	-0.23	0.16	-0.09	-0.36	0.18
> 25 weeks of gestation	-0.12	-0.31	0.07	-0.09	-0.35	0.17
Mean of three urines	-0.21	-0.47	0.06	-0.10	-0.47	0.27
Adjusted [d]						
< 18 weeks of gestation	-0.06	-0.23	0.11	0.14	-0.10	0.38
18 – 25 weeks of gestation	0.04	-0.15	0.23	0.01	-0.26	0.28
> 25 weeks of gestation	-0.02	-0.20	0.16	0.06	-0.20	0.32
Mean of three urines	-0.04	-0.30	0.22	0.13	-0.24	0.50
Mutually adjusted [e]						
< 18 weeks of gestation	-0.07	-0.25	0.11	0.15	-0.10	0.40
18 – 25 weeks of gestation	0.08	-0.13	0.28	-0.03	-0.32	0.26
> 25 weeks of gestation	-0.05	-0.24	0.15	0.05	-0.22	0.32

a. square root transformed.
b. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.
c. Unadjusted model adjusted for season of urine collection.
d. Adjusted for season of urine collection, maternal age, maternal psychopathology (0 = no problems, 1= borderline: GSI score > 80%), sex of the child, ethnicity (Dutch, other-Western and non-Western), education (low, intermediate and high), income (low, middle and high), marital status, maternal alcohol consumption (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasionally alcohol consumption during pregnancy and frequently alcohol consumption during pregnancy), non-verbal IQ of the mother, BMI categories (<18.5, 18.5-25, 25-30, 30+), height (≤168,>168) of the mother, parity categories (0, 1, 2+), and smoking (no smoking during pregnancy, smoked until pregnancy was known, smoked during pregnancy).
e. Adjusted model with the inclusion of the three exposures in one model.

Table S15. Maternal education [a] stratified difference in standardized ADHD score (and 95% confidence interval) across childhood per 10-fold increase in maternal urine dialkyl phosphate metabolite concentrations in nmol/g creatinine.

	ADHD score (N=781)									
	Low (N=125)			Intermediate (N=239)			High (N=417)			
Total dialkyl phosphates [b]	B	95%CI		B	95%CI		B	95%CI		P interaction
None										
< 18 weeks of gestation	-0.02	-0.48	to 0.44	-0.20	-0.54	to 0.13	-0.05	-0.30	to 0.20	0.938
18 – 25 weeks of gestation	-0.05	-0.56	to 0.47	0.08	-0.29	to 0.45	0.06	-0.21	to 0.33	0.751
> 25 weeks of gestation	-0.22	-0.72	to 0.29	-0.16	-0.51	to 0.20	0.15	-0.10	to 0.41	0.099
Mean of three urines	-0.17	-0.85	to 0.51	-0.20	-0.70	to 0.31	0.11	-0.26	to 0.48	0.358
Adjusted [c]										
< 18 weeks of gestation	0.06	-0.40	to 0.52	-0.16	-0.48	to 0.17	-0.08	-0.33	to 0.17	0.496
18 – 25 weeks of gestation	0.15	-0.43	to 0.72	0.10	-0.28	to 0.47	0.09	-0.18	to 0.36	0.967
> 25 weeks of gestation	-0.05	-0.60	to 0.50	-0.07	-0.43	to 0.28	0.13	-0.12	to 0.37	0.549
Mean of three urines	0.10	-0.63	to 0.83	-0.11	-0.62	to 0.40	0.09	-0.28	to 0.46	0.944
Mutually adjusted [d]										
< 18 weeks of gestation	0.02	-0.46	to 0.50	-0.17	-0.51	to 0.16	-0.11	-0.37	to 0.14	0.507
18 – 25 weeks of gestation	0.17	-0.47	to 0.82	0.17	-0.24	to 0.57	0.08	-0.20	to 0.36	0.940
> 25 weeks of gestation	-0.11	-0.71	to 0.49	-0.10	-0.47	to 0.27	0.12	-0.14	to 0.38	0.531

a. Low: no education finished, Primary education, lower vocational training, intermediate general school or <3 years at general. Intermediate: ≥3 years of secondary education, Intermediate vocational training or first year of higher vocational training. High: university degree or higher vocational training.

b. Total Dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.

c. Adjusted for maternal age, maternal psychopathology (0 = no problems, 1= borderline: GSI score > 80%), sex of the child, ethnicity (Dutch, other-Western and non-Western), education (low, intermediate and high), income (low, middle and high), marital status, maternal alcohol consumption (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasionally alcohol consumption during pregnancy and frequently alcohol consumption during pregnancy), non-verbal IQ of the mother, BMI categories (<18.5, 18.5-25, 25-30, 30+), height (≤168,>168) of the mother, parity categories (0, 1, 2+), and smoking (no smoking during pregnancy, smoked until pregnancy was known, smoked during pregnancy).

d. adjusted model with the inclusion of the three exposures in one model.

Table S16. Maternal education [a] stratified difference in autistic traits score (and 95% confidence interval) at age 6 years per 10-fold increase in maternal urine dialkyl phosphate metabolite concentrations in nmol/g creatinine.

	Autistic traits score [b] (N=622)									
	Low (N=78)			Intermediate (N=176)			High (N=368)			
Total dialkyl phosphates [b]	B	95%CI		B	95%CI		B	95%CI		P interaction
None										
< 18 weeks of gestation	0.28	-0.26	to 0.83	0.08	-0.39	to 0.55	0.06	-0.28	to 0.40	0.592
18 – 25 weeks of gestation	0.30	-0.29	to 0.88	0.05	-0.50	to 0.61	-0.12	-0.49	to 0.25	0.262
> 25 weeks of gestation	-0.08	-0.66	to 0.51	0.05	-0.45	to 0.54	0.03	-0.33	to 0.38	0.838
Mean of three urines	0.32	-0.46	to 1.11	0.13	-0.60	to 0.86	-0.01	-0.52	to 0.50	0.509
Adjusted [c]										
< 18 weeks of gestation	0.39	-0.20	to 0.98	0.04	-0.45	to 0.52	0.11	-0.24	to 0.45	0.494
18 – 25 weeks of gestation	0.27	-0.39	to 0.93	-0.08	-0.67	to 0.50	-0.06	-0.43	to 0.31	0.158
> 25 weeks of gestation	0.05	-0.68	to 0.79	0.20	-0.33	to 0.72	-0.07	-0.43	to 0.29	0.297
Mean of three urines	0.46	-0.41	to 1.33	0.13	-0.67	to 0.93	-0.01	-0.53	to 0.51	0.155
Mutually adjusted [d]										
< 18 weeks of gestation	0.37	-0.31	to 1.04	0.05	-0.45	to 0.54	0.13	-0.23	to 0.48	0.490
18 – 25 weeks of gestation	0.12	-0.66	to 0.90	-0.17	-0.79	to 0.45	-0.07	-0.46	to 0.33	0.171
> 25 weeks of gestation	-0.11	-0.90	to 0.68	0.24	-0.31	to 0.80	-0.07	-0.45	to 0.30	0.294

a. Low: no education finished, Primary education, lower vocational training, intermediate general school or <3 years at general. Intermediate: ≥3 years of secondary education, Intermediate vocational training or first year of higher vocational training. High: university degree or higher vocational training

b. square root transformed

c. Total Dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.

d. Adjusted for maternal age, maternal psychopathology (0 = no problems, 1= borderline: GSI score > 80%), sex of the child, ethnicity (Dutch, other-Western and non-Western), education (low, intermediate and high), income (low, middle and high), marital status, maternal alcohol consumption (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasionally alcohol consumption during pregnancy and frequently alcohol consumption during pregnancy), non-verbal IQ of the mother, BMI categories (<18.5, 18.5-25, 25-30, 30+), height (≤168,>168) of the mother, parity categories (0, 1, 2+), and smoking (no smoking during pregnancy, smoked until pregnancy was known, smoked during pregnancy).

e. adjusted model with the inclusion of the three exposures in one model.

Table S17. Associations (and 95% confidence intervals) between log10 transformed child urine dialkyl phosphate metabolite concentrations in nmol/g creatinine at age 6 years and standardized ADHD scores at age 6 and 10 years and autistic traits scores at age 6 years.

		ADHD scores at age 6 years (N=743) [a]			ADHD scores at age 10 years (N=562) [a]			Autistic traits scores at age 6 years (N=597) [a]		
		B	95% CI		B	95% CI		B	95% CI	
Unadjusted										
	Dialkyl phosphates (total) [b]	-0.13	-0.26	to 0.00	-0.06	-0.21	to 0.08	-0.02	-0.18	to 0.13
	Diethyl alkyl phosphates [c]	-0.15	-0.26	to -0.03	-0.05	-0.18	to 0.09	-0.05	-0.19	to 0.09
	Dimethyl alkyl phosphates [d]	-0.10	-0.22	to 0.01	-0.08	-0.22	to 0.05	-0.02	-0.16	to 0.12
Adjusted [e]										
	Dialkyl phosphates (total) [b]	-0.06	-0.19	to 0.06	-0.04	-0.19	to 0.10	0.07	-0.09	to 0.22
	Diethyl alkyl phosphates [c]	-0.10	-0.21	to 0.02	-0.04	-0.17	to 0.09	0.02	-0.12	to 0.16
	Dimethyl alkyl phosphates [d]	-0.04	-0.16	to 0.07	-0.06	-0.19	to 0.07	0.07	-0.07	to 0.20

a. Square root transformed

b. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.

c. Diethyl alkyl phosphates is the sum of DEDTP, DETP, DEP.

d. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP and DMP.

e. Adjusted for averaged maternal DAP concentrations, maternal age, sex of the child, child ethnicity (Dutch, other-Western and non-Western), maternal education (low, intermediate and high), household income at child age 6 years (low, middle, and high), marital status at child age 6 years, age standardized BMI of the child at the age of 6 years, and maternal smoking (no smoking during pregnancy, smoked until pregnancy was known, smoked during pregnancy).

Table S18. Sex stratified difference (and 95% confidence interval) in ADHD score at child age 6 and 10 years and autistic traits score at child age 6 years per 10-fold increase in child urine total dialkyl phosphate metabolite concentrations [a] in nmol/g creatinine measured at age 6 years

	Boys		Girls			
	B	95%CI	B	95%CI	P-value [b]	P-value [c]
ADHD scores at 6 years (N=743) [d]						
Unadjusted dialkyl phosphates (total)	-0.18	-0.36 to -0.01	-0.08	-0.26 to 0.11	0.404	0.404
Adjusted [e] dialkyl phosphates (total)	-0.09	-0.26 to 0.09	-0.03	-0.22 to 0.15	0.649	0.676
ADHD scores at 10 years (N=562) [d]						
Unadjusted dialkyl phosphates (total)	-0.15	-0.36 to 0.06	0.03	-0.18 to 0.24	0.221	0.221
Adjusted [e] dialkyl phosphates (total)	-0.08	-0.29 to 0.13	0.00	-0.20 to 0.21	0.303	0.597
Autistic traits at 6 years (N=597) [d]						
Unadjusted dialkyl phosphates (total)	-0.12	-0.33 to 0.09	0.10	-0.12 to 0.33	0.146	0.146
Adjusted [e] dialkyl phosphates (total)	-0.03	-0.24 to 0.18	0.17	-0.06 to 0.39	0.176	0.205

a. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.
b. P-value for interaction.
c. P-value for interaction based on the augmented product term approach (Buckley et al. 2017).
d. Square root transformed.
e. Adjusted for maternal age, sex of the child, child ethnicity (Dutch, other-Western and non-Western), maternal education (low, intermediate and high), household income at child age 6 years (low, middle, and high), marital status at child age 6 years, age standardized BMI of the child at the age of 6 years, and maternal smoking (no smoking during pregnancy, smoked until pregnancy was known, smoked during pregnancy).

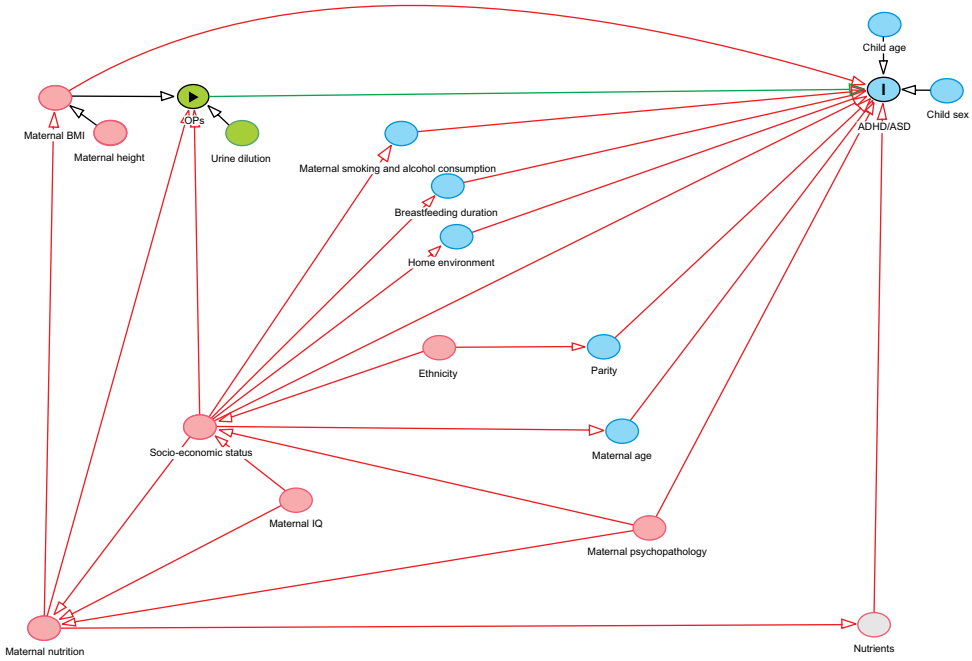

Figure 1. Directed Acyclic Graph of the prenatal organophosphate pesticides and = Attention-Deficit/Hyperactivity Disorder and autistic traits association. Potential adjustment variables were selected a priori defined with a Directed Acyclic Graph (DAG) using the Dagitty software (Textor et al. 2017). The DAG was based on previous studies of OP pesticides and child neurodevelopment and on biologically plausible covariate–exposure and covariate–outcome associations observed in our data. Green circles represent ancestors of the exposure, blue circles ancestors of the outcome, pink circles ancestors of both exposure and outcome, and gray circles represent unobserved variables. ADHD/ASD= Attention-Deficit/Hyperactivity Disorder and autistic traits in children. OPs= Prenatal exposure to organophosphate pesticides. Maternal BMI: Maternal body mass index. Maternal nutrition: Fruit intake. Maternal SES= maternal socio-economic status: maternal education, household income and marital status. Child age: Child age at assessment. Home environment: Infant/Toddler Home Observation for Measurement of the Environment.

Part IV

The effect of exposure to non-persistent chemicals on potential mediators of the association with neurodevelopment

6

Chapter 6

Organophosphate pesticides exposure in pregnant women and maternal and cord blood thyroid hormone concentrations

Mulder, T. A., van den Dries, M. A., Korevaar, T., Ferguson, K. K., Peeters, R. P., & Tiemeier, H. (2019).

Environment international, 132, 105124.

Abstract

Background: Animal studies suggest that organophosphate (OP) pesticides exposure affects thyroid function, but evidence in humans remains sparse and inconclusive. Gestational exposure is of particular interest, since thyroid hormone is essential for fetal brain development. OP pesticides are able to cross the placental and blood-brain barrier and may interfere with fetal development processes regulated by thyroid hormone.

Objective: To investigate the association of gestational OP pesticides exposure during pregnancy with maternal and cord blood thyroid hormone concentrations.

Methods: This study was embedded within Generation R (Rotterdam, the Netherlands), a prospective population-based birth cohort. Mother-child pairs with OP pesticides assessment and maternal (N = 715) or cord blood (N = 482) thyroid hormone measurements were included. OP pesticides exposure was assessed at <18, 18–25, and >25 weeks gestation by measuring six urinary dialkylphosphate (DAP) metabolites. Thyroid stimulating hormone (TSH) and free thyroxine (FT4) were measured in maternal and cord blood. Maternal measures also included total thyroxine (TT4) and TPO antibodies (TPOAbs). To study the association of creatinine-adjusted DAP metabolite concentrations with thyroid function and TPO antibodies, multivariable linear regression models including relevant confounders were used.

Results: There was no association of DAP metabolites with maternal TSH, FT4, TT4 or TPOAb concentrations during pregnancy. Similarly, there was no association of DAP metabolites with cord blood TSH or FT4. Results did not change when DAP concentrations were analyzed at individual time points or as mean gestational exposure.

Conclusion: Gestational OP pesticides exposure, as assessed by repeatedly measured urinary DAP metabolite concentrations in an urban population, was not associated with maternal or cord blood thyroid hormone concentrations. These findings do not support a mediating role for serum thyroid hormone availability in the relation of early life exposure to low levels of OP pesticides with child neurodevelopment. However, disruption of the thyroid system at tissue level cannot be excluded. In addition, this is one of the first studies on this subject and measurement error in DAP metabolites might have resulted in imprecise estimates. Future studies should use more urine samples to increase precision and should investigate specific OP pesticide metabolites.

Introduction

Organophosphate (OP) pesticides are widely applied for pest control in agriculture worldwide, which leads to widespread exposure of the general population to low levels of OP pesticides.[1] Some populations are occupationally exposed, but the exposure of pregnant women most likely occurs through their diet.[2-5] Importantly, OP pesticides can cross the placental and blood-brain barriers and may interfere with optimal fetal development at different levels.[6] Fetal growth and differentiation of almost all tissues, including the brain, adipose tissue and bone is regulated by thyroid hormones.[7] During pregnancy, major changes occur in thyroid physiology in order to provide sufficient hormones to both the mother and fetus. Since the fetal thyroid gland is not fully mature before 20 weeks of gestation, the fetus largely depends on the supply of maternal thyroxine during early pregnancy.[8] The increase in thyroid hormone binding globulin and thyroid hormone degradation due to placental expression of deiodinase type 3 requires an increased production of maternal thyroid hormones.[9] This increased demand suggests that pregnancy may be a vulnerable period for potential thyroid disruption by different stressors, including environmental chemical exposures.

The association of OP pesticides with thyroid hormone on fetal neurodevelopment is of specific interest. Thyroid hormones play a major role in neuronal cell proliferation, migration, and differentiation.[10,11] High exposure levels of OP pesticides can have neurotoxic effects by inhibiting acetylcholinesterase. Animal studies have shown that exposure at levels below the threshold for acetylcholinesterase inhibition can also adversely affect thyroid hormone-dependent neurodevelopment.[12,13] Both prenatal thyroid hormone shortage and exposure to OP pesticides have been associated with adverse neurobehavioral and birth outcomes in children.[14-18] Therefore, it has been hypothesized that disruption of thyroid function is a potential mechanism relating prenatal OP pesticides exposure to brain development.[19]

Results from animal studies suggest that organophosphates might interfere with thyroid function, although findings are inconclusive. Some studies in adult animals report an increased[20] or decreased[21] serum total thyroxine (TT4) concentration after exposure to dimethoate or malathion, respectively, whereas chlorpyrifos-methyl, malathion, or monocrotophos did not affect TT4 concentrations.[22-25] Findings for thyroid stimulating hormone (TSH) and thyroid peroxidase (TPO) are also mixed. Serum TSH concentrations were found to be lower after exposure to monocrotophos, [25] but did not differ after exposure to dimethoate or chlorpyrifos-methyl.[20,22]

Studies in humans are equally inconclusive, scarce, and do not specifically test windows of vulnerability such as pregnancy. Another gap is that studies in humans did not utilize

repeated measurements of OP pesticide exposure.[26] Analyzing multiple urine specimens per subject is of importance, because OP pesticides are known to have a short half-life, which can result in substantial day-to-day variability within subjects.[27] In addition, repeated measurements of OP pesticides during pregnancy enable the investigation of potential developmental windows of vulnerability. While one study in pregnant women in China found that higher gestational OP pesticide exposure, as measured by urinary dialkylphosphate (DAP) metabolite concentrations, is associated with higher FT4 and lower TSH at hospital admission for delivery,[28] all other studies but one have been performed in adult males and reported contradictive results.[26] Higher urinary DAP metabolite concentrations were associated with higher TT4 and TSH in occupationally exposed males.[29] In contrast, higher urinary levels of 5,6-trichloro-2-pyridinol (TCPy), a metabolite of chlorpyrifos, were associated with lower FT4 and higher TSH concentrations in males visiting an infertility clinic.[30] Furthermore, higher urinary TCPy levels were associated with lower FT4 and TSH in males from the general population, whereas TCPy levels were associated with higher TSH among women > 60 years of age only.[31] No study investigated the association of maternal OP pesticides exposure during pregnancy with thyroid function of their offspring.

To address these inconsistencies and research gaps, we investigated the association of repeatedly measured gestational OP pesticides exposure with maternal and cord blood thyroid hormone concentrations in an urban population with relatively high exposure levels compared to those observed in most other birth cohorts.[32-35] We hypothesized that higher OP pesticide exposure would be associated with lower FT4 and higher TSH concentrations.

Methods

Participants

This study was embedded in Generation R, a prospective population-based cohort from fetal life onwards.[36] Eligible participants were pregnant women living in Rotterdam, the Netherlands, with an expected delivery date between April 2002 and January 2006. Mothers were enrolled during pregnancy or in the first months after the birth of their child when newborns attended child health centers for routine visits. The baseline participation rate was estimated at 61%. Of the 9,778 mothers who participated in the study, 8,879 (91%) were enrolled during pregnancy. Between February 2004 and January 2006, spot urine specimens were collected during early, middle, and late pregnancy (<18, 18–25, >25 weeks of gestational age, respectively) at the time of routine ultrasound examinations when in total 4,918 women were enrolled. Of these, 2,083 women provided a complete set of three urine specimens. Of the women with a singleton pregnancy and follow-up

data including neurobehavioral, sociodemographic and health data on the offspring (n=1,449), 800 were randomly selected for measurements of urinary dialkylphosphate (DAP) metabolites.[5] Of these, 784 had sufficient urine volume for analyses. Of those, 730 women had TSH or FT4 measurements available. Of the offspring, 490 had cord blood TSH or FT4 measurements available. We excluded women with a known thyroid disorder (n = 10 and n = 5, respectively). Most of the women with a known thyroid disorder received thyroid (interfering) medication including levothyroxine during pregnancy (n = 9 and n = 4, respectively). In addition, we excluded women who had undergone in vitro fertilization (n = 5 and n = 3, respectively) (Fig. 1). The Medical Ethics Committee of the Erasmus Medical Centre, Rotterdam, approved the study and written informed consent was obtained from all parents.

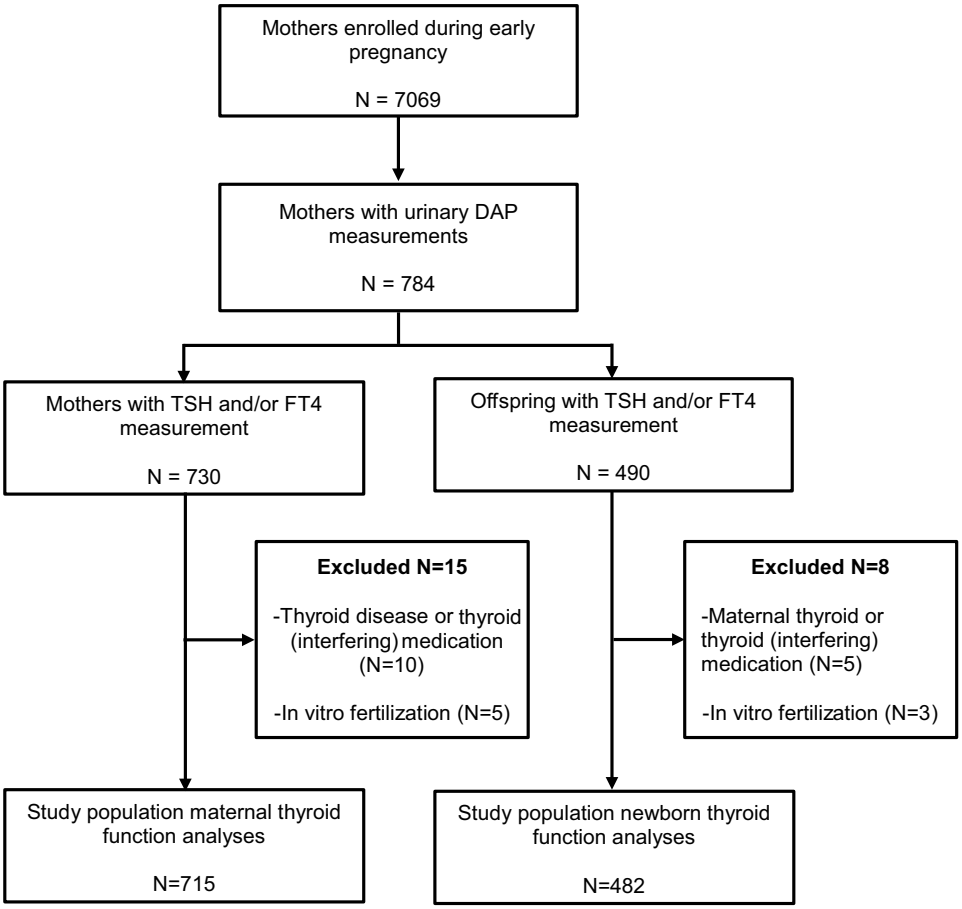

Figure. 1. Flowchart of the study population.

Urine collection and analysis of DAP metabolites

Measurements of six non-specific DAP metabolites of OP pesticides were conducted at Institut National de Santé Publique (INSPQ) in Quebec, Canada, using gas chromatography coupled with tandem mass spectrometry (GC–MS/MS).[37] Three dimethyl (DM) metabolites (dimethylphosphate (DMP), dimethylthiophosphate (DMTP), and dimethyldithiophosphate (DMDTP)) were determined, as well as three diethyl (DE) metabolites (diethylphosphate (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP))). The limit of detection (LOD) was 0.26 µg/L for DMP, (0% < LOD), 0.40 for DMTP, (2–4% < LOD), 0.09 for DMDTP, (18–20% < LOD), 0.50 for DEP, (3–5% < LOD), 0.12 for DETP (12% < LOD) and 0.06 for DEDTP (81–85% < LOD). Measured concentrations below the LOD were included in the data analysis as the original values determined by the GC–MS/MS. The inter-day precision of the method, expressed as the coefficient of variation (CV%), varied between 4.2 and 8.8 for DEDTP, 4.1–7.2 for DEP, 5.0–9.1 for DETP, 5.5–7.1 for DMDTP, 5.3–8.0 for DMP and 5.5–7.7 for DMTP based on reference materials (clinical check-urine level II 637 E-495 and MRM E-459) [5]. To account for urinary dilution, OP pesticide metabolite concentrations were divided by urinary creatinine concentrations, which were determined based on the Jaffe reaction.[38] The ranges (min-max) for measured creatinine concentrations in early, middle and late pregnancy were 0.03–3.97, 0.04–4.29, and 0.07–4.96, respectively. A more detailed description of the urine collection and analysis of DAP metabolites can be found elsewhere.[5,39]

Thyroid function measurements

Maternal serum samples were obtained in the first half of pregnancy (<18 weeks, mean 12.9 weeks, SD 1.81, 95% range 9.8–17.1). These samples were collected concurrently with the spot urine specimens obtained during the first urine collection phase. Cord blood samples were obtained directly after birth (mean 40.3 weeks, SD 1.32, 95% range 37.1–42.3). Maternal reference ranges were 0.03–4.04 mU/L for TSH, 10.4–22.0 pmol/L for FT4, and 96.0–219.0 nmol/L for TT4. Cord reference ranges were 3.41–33.80 mU/L for TSH and 15.3–28.1 pmol/L for FT4. Plain tubes were centrifuged and serum was stored at –80 °C. TSH, FT4, and TT4 concentrations in maternal and cord blood serum samples were determined using chemiluminiscence assays (Vitros ECI; Ortho Clinical Diagnostics). The intra- and interassay coefficients of variation were <4.1% for TSH, <5.4% for FT4 and <6.4% for TT4. TPOAbs were measured only in maternal blood using the Phadia 250 immunoassay (Phadia AB, Uppsala, Sweden) and considered positive when >60 IU/ml.[40]

Covariates

Potential confounders were selected a priori based on previous studies of OP pesticides and thyroid function.[26,28] Gestational age at blood sampling and child sex were included as

independent predictors of thyroid function. Information on maternal age at enrollment, ethnicity, parity (0, 1, or >1), and smoking behavior (no smoking during pregnancy, smoked until pregnancy recognized, and continued smoking during pregnancy), and fruit intake was obtained through postal questionnaires filled in during pregnancy. Maternal ethnicity was based on parent's country of birth and considered Dutch when both parents were born in the Netherlands, and non-Dutch if one parent was born outside the Netherlands. This categorization was defined according to the classification of Statistics Netherlands.[41] Fruit intake was assessed in the first trimester using a modified version of a validated food frequency questionnaire and was adjusted for energy intake. Body mass index (BMI) was calculated using length and weight as measured at study enrollment. Urinary iodine concentration and creatinine concentration were measured in spot urine samples in early and middle pregnancy at the time of OP pesticide metabolites measurements. The urinary iodine-to-creatinine ratio (UICr) was used as a measure of iodine status.[42,43]

Midwives and hospital registries provided information on child sex and gestational age at birth. Maternal gestational age at blood and urine sampling was defined using ultrasound measurements of crown-rump length or biparietal diameter, using dating curves derived from this cohort.[44] Except for smoking (8%) and fruit intake (22%), missing data on covariates were all <1%.

Statistical analyses

For each urine collection phase, the three DM metabolites (nmol/L) were summed as total DM and the three DE metabolites (nmol/L) were summed as total DE. Total DAP concentrations (nmol/L) per urine collection phase were calculated by summing the six metabolites. Urinary DAP concentrations were expressed on a volume and creatinine basis to control for urine dilution (nmol/g creatinine). The geometric means of the total DAP, DM, and DE concentrations at three different time points were calculated to get an estimate of OP pesticides exposure across pregnancy. Subsequently, the geometric means were log10 transformed in order to reduce the influence of outliers and to have a better model fit. In addition, log transformation of the DAP concentrations improves comparability since previous studies have used log transformed DAP concentrations.[28,29] To approach normality, TSH values were also log10 transformed.

We used multivariable linear regression models to study the association of OP pesticide metabolites with maternal (TSH, FT4, and TT4) and cord blood (TSH and FT4) thyroid hormone concentrations. In the analyses of maternal thyroid function, we only studied DAP metabolite concentrations measured concurrently (<18 weeks gestation) with thyroid function. DAP concentrations at individual time points or as the mean of the three measurements were studied as determinants of cord blood thyroid hormones. Assumptions of linear regression models, including linearity, homoscedasticity, and normal

distributions of the model residuals, were assessed with residual plots, Q-Q plots and histograms and were met for all models.

All models were adjusted for gestational age at blood sampling, and additionally adjusted for maternal age, parity, smoking and ethnicity, and child sex. When the association between metabolite concentrations at individual time points and thyroid function was tested, models were additionally adjusted for creatinine, gestational age at urine sampling, and season of urine collection.

Effect modification by child sex was explored by introducing a product interaction term of DAP concentration and child sex to the model with cord blood TSH or FT4 as outcome. A P-value below 0.05 was used for interaction terms to screen for effect modification.

Several additional analyses were performed. First, the association of DM, DE, and DAP concentrations with TPOAb positivity was analyzed with the use of logistic regression models by using two cut-offs for TPOAb positivity: 60 and 20 IU/ml. We did not only use the assay-specific cut-off of 60 IU/ml but ran analyses using the latter, since the manufacturer cut-off for TPOAb positivity may fail to identify women with TPOAb concentrations sufficient to affect thyroid function. In Generation R, higher mean TSH and lower FT4 concentrations were already observed for TPOAbs >26 IU/ml.[45] Second, the analyses on maternal thyroid function were repeated in TPOAb negative women only to rule out confounding and effect modification by TPOAb positivity. Third, models were additionally adjusted for maternal BMI, UICr, TPOAbs, or fruit intake. We adjusted for maternal UICr at the time of thyroid function measurement in models with maternal thyroid function, or for the average maternal UICr from two time points during pregnancy in models with cord blood thyroid hormone concentrations. Maternal BMI might be a determinant as well as a consequence of thyroid function.[46,47] TPOAbs might be on the OP pesticides – thyroid function pathway. In addition, UICr and TPOAbs affect thyroid function but are less likely to affect DAP metabolite concentrations. Therefore, maternal BMI, UICr, and TPOAbs were not included in the main analyses. Fruit intake was used as a proxy for a healthy diet including micronutrients important for a normal thyroid function. Fourth, effect modification by UICr or TPOAbs was tested by introducing a product interaction term of DAP concentration and UICr or TPOAbs to the models. Fifth, we refitted models with metabolite concentrations expressed as nmol/L without correction for creatinine, as very high or low creatinine values might influence the results. Sixth, we used inverse probability weighting to correct for loss to follow-up and to account for potential selection bias because participants in our study sample were more likely be older, have a lower BMI, a higher level of education, and a higher income compared to the full cohort (Table S5).Missing covariate and DAP metabolite data were imputed 10 times with the Multivariate Imputation by Chained Equations (MICE) method in

R.[48,49] DAP metabolite concentrations were log10 transformed prior to the multiple imputation procedure to approach normality. Thyroid function variables were included as predictors for the imputation, but were not imputed. DAP metabolite concentrations, thyroid function parameters and all covariates indicated above were used to impute missing data. In addition, variables likely to be associated with these covariates or shown to be associated with DAP metabolite concentrations were used to impute missing data.[5] These included birthweight, marital status, household income, and maternal education. All statistical analyses were performed with R statistical software version 3.3.2. using a 2-sided significance level of $P < 0.05$.

Results

The final study population consisted of 715 pregnant women and 482 newborns (Fig. 1). Descriptive statistics are shown in Table 1. Mean differences between DAP concentrations across time points were modest. The intra class correlations (estimated by using a 2-way mixed-effects model with absolute-agreement) for DAP metabolite concentrations varied between 0.22 and 0.26 for a single-measurement and between 0.48 and 0.52 for the mean of the three measurements (Table S1).

Median gestational age at first DAP measurement and serum thyroid measurements was 12.9 weeks (95% range 9.8–17.1) in pregnant women. Cord blood samples were obtained directly after birth at median gestational age 40.3 weeks (95% range 37.1–42.3). The women had a median TSH concentration of 1.31 mU/L and a median FT4 concentration of 14.6 pmol/L. We observed TPOAb positivity in 42 (5.9%) women. The neonatal median TSH and FT4 concentrations were 9.43 mU/L and 20.9 pmol/L, respectively. In non-response analyses, maternal and cord blood TSH and FT4 concentrations did not meaningfully differ between women grouped on the basis of organophosphates data availability. However, women included in the analyses were more often Dutch, had a higher mean age, a lower mean BMI, and a higher mean fruit consumption (Table S10).

There was no association of DM, DE, or DAP concentrations measured concurrently with thyroid function with maternal TSH, FT4 or TT4 in adjusted models (Table 2). For example, a 10-fold higher total DAP concentration at the time of thyroid function measurement was not associated with TSH (β [95% CI]: 0.00 [–0.04 to 0.04]). The results were similar after the exclusion of TPOAb positive women (Table S2). In addition, there was no association of DM, DE or DAP measured concurrently with thyroid function and TPOAb positivity, irrespective of the cut-off chosen (Table S3). Finally, DM, DE, or DAP concentrations measured during early, mid, and late pregnancy (Table S4) or as mean of the three measurements (Table 3) were not associated with cord blood TSH or. There

Table 1. Descriptive characteristics of Mother and Child Pairs

	Maternal thyroid hormones available (n=715)		Cord blood thyroid hormones available (n=482)	
Characteristic	Median	(95% range)[c]	Median	(95% range)[c]
Gestational age at urine sampling, weeks				
Urine collection phase 1	12.9	(9.8-17.1)	12.9	(9.6-17.1)
Urine collection phase 2	20.4	(18.9-22.8)	20.4	(18.9-22.4)
Urine collection phase 3	30.2	(28.9-32.5)	30.2	(28.9-32.5)
Gestational age at blood sampling, weeks	12.9	(9.8-17.1)	40.3	(37.1-42.3)
Maternal age, years	31.0	(20.4-38.9)	30.9	(20.2-38.7)
Maternal BMI, Kg/m2	23.1	(18.7-35.6)	23.0	(18.3-34.6)
Parity[a]				
0	442	61.8	296	61.4
1	190	26.6	53	11.0
≥2	83	11.6	67	13.9
Smoking[a]				
No smoking during pregnancy	543	75.9	370	76.8
Until pregnancy recognized	71	9.9	48	10.0
Continued during pregnancy	101	14.1	64	13.3
Ethnicity[a]				
Dutch	410	57.3	283	58.7
Morrocan	38	5.3	25	5.2
Turkish	40	5.6	26	5.4
Surinamese	63	8.8	43	8.9
Other western	92	12.9	61	12.7
Other non-western	72	10.1	44	9.1
Child sex[a] (girls %)	353	49.4	234	48.5
Dimethyl metabolites, nmol/g creatinine				
Urine collection phase 1	239.8	(56.2-1178.8)	256.0	(54.2-1368.6)
Urine collection phase 2	269.1	(59.6-1225.1)	268.1	(61.3-1260.8)
Urine collection phase 3	249.6	(59.0-1037.8)	249.3	(55.6-1083.5)
Diethyl metabolites, nmol/g creatinine				
Urine collection phase 1	42.8	(7.3-258.8)	42.5	(7.9-267.4)
Urine collection phase 2	41.5	(6.6-260.2)	41.5	(7.5-282.0)
Urine collection phase 3	41.1	(6.4-219.1)	41.1	(7.9-246.7)
Total dialkylphosphate metabolites, nmol/g creatinine				
Urine collection phase 1	306.0	(66.5-1363.5)	317.5	(66.0-1531.8)
Urine collection phase 2	317.7	(79.0-143.5)	316.1	(84.9-1346.3)
Urine collection phase 3	305.5	(71.7-1124.6)	307.5	(71.7-1223.0)
Average dialkylphosphate metabolites across pregnancy, nmol/g creatinine				
Dimethyl metabolites	259.5	(81.8-686.8)	259.1	(83.7-698.5)
Diethyl metabolites	42.6	(9.8-144.6)	43.2	(11.4-148.3)
Total dialkylphosphate metabolites	311.6	(97.0-800.5)	312.0	(102.2-813.7)
TSH, mU/L	1.31	(0.0-4.24)	9.43	(2.78-36.67)
FT4, pmol/L	14.6	(10.3-21.9)	20.9	(15.6-32.1)
TPOAb positivity[a, b]	42	5.9	.	.
Urinary iodine to creatinine ratio, µg/g				
Urine collection phase 1	207.0	(70.3-561.7)	203.3	(73.7-538.4)
Urine collection phase 2	231.5	(67.4-568.6)	229.3	(75.5-563.0)

Data are shown after multiple imputation (see methods section) and are extracted from the 10th imputed dataset.
a Data shown as n (%).
b Considered positive when >60 IU/ml.
c 5th and 95th percentile.

Table 2. Associations between dialkyl phosphate metabolite concentrations and maternal thyroid function during pregnancy

	Mean TSH		Mean FT4		Mean TT4	
<18 weeks gestation	β (95% CI)	*p*	β (95% CI)	*p*	β (95% CI)	*p*
Total dialkyl phosphates						
Model 1	0.00 (-0.04 to 0.04)	0.95	0.33 (-0.32 to 0.97)	0.32	-2.33 (-9.03 to 4.38)	0.50
Model 2	0.00 (-0.04 to 0.04)	0.92	0.36 (-0.27 to 0.99)	0.27	-1.24 (-7.83 to 5.35)	0.71
Dimethyl alkyl phosphates						
Model 1	0.01 (-0.03 to 0.04)	0.79	0.20 (-0.41 to 0.81)	0.51	-2.66 (-9.03 to 3.71)	0.41
Model 2	0.00 (-0.04 to 0.04)	0.90	0.25 (-0.35 to 0.85)	0.41	-1.97 (-8.23 to 4.29)	0.54
Diethyl alkyl phosphates						
Model 1	-0.01 (-0.04 to 0.02)	0.42	0.28 (-0.19 to 0.75)	0.24	-0.27 (-5.16 to 4.62)	0.91
Model 2	-0.02 (-0.05 to 0.01)	0.30	0.27 (-0.19 to 0.72)	0.25	0.73 (-4.02 to 5.81)	0.76

All dialkyl phosphate metabolite concentrations (nmol/g creatinine) and TSH values were log transformed.
N= 710 for TSH, n=709 for FT4, and n=712 for TT4.
Model 1 was adjusted for gestational age at blood sampling and creatinine.
Model 2 was additionally adjusted for maternal age, parity, smoking and ethnicity, child sex, and season.

Table 3. Associations between dialkyl phosphate metabolite concentrations during pregnancy and cord blood thyroid hormone concentrations

	Mean TSH		Mean FT4	
Averaged across pregnancy	β (95% CI)	*p*	β (95% CI)	*p*
Total dialkyl phosphates				
Model 1	-0.07 (-0.17 to 0.03)	0.16	0.13 (-1.43 to 1.69)	0.87
Model 2	-0.07 (-0.18 to 0.03)	0.15	0.28 (-1.35 to 1.90)	0.74
Dimethyl alkyl phosphates				
Model 1	-0.07 (-0.17 to 0.02)	0.13	0.06 (-1.44 to 1.56)	0.94
Model 2	-0.07 (-0.17 to 0.02)	0.14	0.16 (-1.38 to 1.71)	0.84
Diethyl alkyl phosphates				
Model 1	-0.02 (-0.10 to 0.06)	0.64	0.29 (-0.95 to 1.53)	0.64
Model 2	-0.03 (-0.11 to 0.05)	0.43	0.40 (-0.89 to 1.68)	0.55

Average dialkyl phosphate metabolite concentrations (nmol/g creatinine) were computed by the geometric mean of the three urine collection phases.
All dialkyl phosphate metabolite concentrations and TSH values were log transformed.
N= 472 for TSH and n=477 for FT4.
Model 1 was adjusted for gestational age at blood sampling.
Model 2 was additionally adjusted for maternal age, parity, smoking and ethnicity, and child sex.

was no effect modification of the association between DAP metabolite concentrations and cord blood TSH or FT4 by child sex (data not shown).

Additional adjustment for maternal BMI, UICr, TPOAbs, fruit intake, or inverse probability attrition weights did not change the results for maternal or cord blood thyroid hormones (Table S6 and S7). UICr and TPOAbs did not modify the association between DAP metabolite concentrations and maternal or cord blood thyroid hormone concentrations). Finally, DM, DE, and DAP concentrations were not associated with maternal or cord blood thyroid hormones when metabolite concentrations were expressed as nmol/L without correction for creatinine (Table S8 and S9).

Discussion

In the current study, gestational OP pesticide exposure, as assessed by urinary DAP metabolite concentrations, was not associated with maternal or cord blood thyroid hormone concentrations. DAP concentrations measured concurrently with thyroid function were not associated with maternal TSH, FT4, TT4 or TPOAb concentrations. Similarly, DAP concentrations at individual time points or average DAP concentrations across pregnancy were not associated with cord blood TSH or FT4.

We were able to study the association of gestational OP pesticides exposure with maternal and cord blood thyroid hormone concentrations using a large dataset with repeated measurements of exposure biomarkers and detailed data on potential confounders. Analyzing multiple urine specimens per subject is of importance, because the urinary concentration of DAP metabolites reflects only recent exposure, and individual exposure differs substantially from day-to-day, depending on diet.[3,27] The estimation of gestational OP exposure improves if multiple urine specimens across pregnancy are collected from a subject during multiple periods across pregnancy.

Our findings are generalizable to urban populations but cannot be generalized to occupationally exposed individuals for different reasons. First, occupational exposure might involve different OP pesticides resulting in different toxic oxon derivatives after bioactivation. Second, occupational exposure routes might be different compared to our study population. The Generation R population lives in urban settings, where the main route of exposure is through the ingestion of food, and most likely fruits.[5] Third, occupational exposure may result in higher exposure levels. However, DAP metabolite concentrations were 2–3 times higher in our study (311 nmol/g creatinine, 224 nmol/L) than those reported in other birth cohorts from Canada, the United States, and European

countries.[32-35] The relatively high DAP concentrations in our study may be related to the high consumption of fruits and the intense farming practices in the Netherlands.[5]

DAP concentrations in our study are comparable to those observed in a Chinese birth cohort study (270 nmol/g creatinine), which found that higher DAP concentrations were associated with higher FT4 and lower TSH.[28] Importantly, total DM metabolite concentrations in our study were higher (259 vs 186 nmol/g creatinine), whereas total DE metabolite concentrations were lower (43 vs 84 nmol/g creatinine) than in the Chinese birth cohort. Although still being detected, several OP pesticides were banned in or before 2006 in the Netherlands, but are still in use in China.[50,51] These include diazinon and phosalone (both OP pesticides that generate DE metabolites) and dichlorvos, naled, fenitrothion, oxydemeton-methyl, and temephos (all OP pesticides that generate DM metabolites). In addition, methamidophos, parathion-methyl, and parathion are banned since 2007 for agricultural use in China, but could still be detected in vegetables.[52] Other OP pesticides types and mixtures as well as the assessment of DAP metabolite concentrations and maternal thyroid hormones on the day of hospital admission for delivery only, while maternal thyroid function samples were obtained before 18 weeks of gestation in our study, best explain why the results in the Chinese birth cohort study are not in line with our findings.

Previous studies in humans were mainly conducted in adult men and reported positive associations of OP pesticides exposure with TT4[29] and TSH,[29,30] and negative associations with FT4[30,31] and TSH.[31] These results are not comparable to those obtained in women during pregnancy because of sex-selective effects of OP pesticides. Rodent studies show higher vulnerability to chlorpyrifos exposure in males compared to females with respect to thyroid function.[22,53] A large cross-sectional study found no association between ever use of OP pesticides by males with the risk of hypo- and hyperthyroidism among their female spouses, whereas organochlorine insecticides and fungicides were associated with thyroid disease in these women.[54] Prior studies assessed OP pesticides exposure only once and only one study focused on OP pesticide exposure during pregnancy.[28-31] To the best of our knowledge, this is the first study investigating maternal OP pesticide exposure during pregnancy and cord blood thyroid hormones using repeated measurements of DAP concentrations.

Importantly, cord blood concentrations of TSH and FT4 reflect fetal thyroid function to a different extent. Since trans placental transfer of TSH is poor,[55] cord blood TSH concentrations reflect fetal thyroid function. In contrast, serum T4 could be detected in cord blood of neonates without a functional thyroid, indicating trans placental transfer of T4 during late gestation.[56] Although transfer of FT4 from mother to fetus decreases throughout pregnancy alongside an increased production of fetal thyroid hormones,[8]

FT4 concentrations in cord blood do not only reflect fetal thyroid function, but maternal thyroid function as well. Therefore, although not very likely, an association between OP pesticides and fetal FT4 concentrations could have been missed.

Our findings are not in line with results from animal studies, which show that gestational OP pesticides exposure can interfere with maternal and newborn thyroid function.[53,57] This discrepancy may suggest that OP pesticides exert different effects on human or animal thyroid function. However, these animal studies are inconsistent, showing opposing effects on thyroid hormone physiology. Some studies in adult animals report an increased[20] or decreased[21] serum total thyroxine (TT4) concentration after exposure to dimethoate or malathion, respectively, whereas chlorpyrifos-methyl, malathion, or monocrotophos did not affect TT4.[22-25] Findings for thyroid stimulating hormone (TSH) and thyroid peroxidase (TPO) are also mixed. Serum TSH concentrations were found to be lower after exposure to monocrotophos,[25] but no differences in serum TSH concentrations were observed after exposure to dimethoate or chlorpyrifos-methyl.[20,22] Our results cannot be directly compared to animal studies for different reasons. First, our study population may have been exposed to a mixture of multiple OP pesticides, whereas animal studies investigate the effects of specific OP pesticides. DAP metabolites are non-specific and cannot be traced back to individual pesticides.[58] The dimethyl OP pesticide dimethoate was the organophosphate most frequently used in 2004 in the Netherlands, whereas other OP pesticides investigated in animal studies were not or less frequently applied on food crops (e.g. chlorpyrifos and malathion).[5] Thus, the DAP metabolites in our population likely do not reflect the same organophosphates used in animal studies. However, we must be cautious because residues of OP pesticides that were banned during the urine collection of our study (2004-2006) were being detected on fruit and vegetables.[50] Second, animals were exposed to different concentrations of OP pesticides compared to our study population. For example, the minimal doses used to study the effects of chlorpyrifos and dimethoate on thyroid function in rodents were 1mg/kg and 2 mg/kg, respectively.[20,53,57] The acceptable daily intake (ADI) concentration for those organophosphates are 0.01 and 0.002 mg/kg in humans.[59] Although the exact exposure levels of our study population are unknown, it is likely that these are similar to or below the ADI and thus much lower than those used in animal studies.

Our findings do not preclude disruption of the thyroid system at tissue level. Thyroid hormones were measured in serum and this does not provide information about thyroid hormone availability or effects in specific tissues. Interestingly, an in vitro study showed that the diethyl OP pesticide malathion can competitively bind to the thyroxine transporter transthyretin.[60] Because TTR is highly expressed in the placenta and brain, OP pesticides might affect thyroid hormone action in these tissues specifically. Moreover, results from

a study in goldfish indicate that OP pesticides might have tissue-specific effects by differentially affecting the three types of deiodinases.[24]

In our study population, OP pesticides exposure during pregnancy most likely occurs through diet, with fruit intake being the main source of exposure.[5] A healthy diet including many fruits, vegetables, nuts, and fish is therefore not only a source of beneficial micronutrients such as selenium, iodine and iron, but can also be a source of OP pesticides. These micronutrients are important for an adequate thyroid function and might counteract the adverse effects of OP pesticide exposure.[61] Therefore, negative confounding by these micronutrients could have attenuated the association between OP pesticide exposure and thyroid function in the current study. However, results did not change when we adjusted our analyses for fruit intake as a proxy for a healthy diet. Moreover, iodine might act as an effect modifier in the association of OP pesticides and thyroid function, since iodine deficient populations may be more vulnerable to thyroid disrupting chemicals.[62] Our study was performed in an iodine-sufficient area, which may mitigate the effects of OP pesticides on thyroid function.[63] However, our study provides no evidence for effect modification by iodine concentration.

This study was limited by the fact that DAP metabolites were used as a proxy for OP pesticide exposure instead of measuring OP pesticides exposure directly. Since preformed DAP metabolites are present in foods and the environment,[64,65] the extent to which DAP metabolite concentrations reflect exposure to the active parent pesticide rather than to less toxic metabolites remains unclear.[66] Yet, the estimation of urinary DAP metabolite concentrations is considered a non-invasive and useful biomarker for OP pesticides exposure[67] and is therefore the most-used method of estimating exposure to this class of compounds in general populations.[68] Moreover, no information was available about the exact time of day of spot urine sampling. The samples include both first morning and random spot samples, since the urine spot samples were collected between 8 am and 8 pm. Concentrations of chemicals, urine volume, and the rate of excretion vary with fluid intake, time of day, and other factors.[69-71] However, time of sample collection is unlikely to confound the association between OP pesticide exposure and thyroid function, since FT4 does not display a circadian rhythm and TSH only varies clearly from day to night.[72] Another limitation of this study is that creatinine concentrations vary during pregnancy. Sensitivity analyses without creatinine correction yielded slightly different results with regards to the direction of some associations. However, all associations remained non-significant.

Although this study used three measurements of DAP metabolite concentrations, it would be ideal to collect urine samples more often during pregnancy. Intraclass correlation coefficients of DAP metabolites were modest in this study. Future studies should use

more urine samples to increase precision of OP pesticide exposure during pregnancy. Chemiluminiscence assays were used to measure FT4 concentrations. These assays may not adequately measure FT4 concentrations due to a rise in thyroid hormone binding proteins during pregnancy.[73] However, this increase in proteins mainly occurs in the third trimester of pregnancy, whereas FT4 concentrations were measured in the first half of pregnancy in the current study. TT4 concentrations were also measured, which are not affected by binding protein interference and results for TT4 were also not significant.

The current study provides no evidence for an association of gestational OP pesticides exposure with maternal or cord blood thyroid hormone concentrations. Since OP pesticides exposure is widespread among pregnant women, our findings are important on a population level. These findings suggest that, contrary to some hypotheses, associations of gestational OP pesticides exposure with neurodevelopment are not mediated by thyroid function. However, we cannot preclude thyroid disruption at the tissue level and our results strictly apply to urban populations and not to occupationally exposed individuals.

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Supplemental Material

Table S1. Intraclass correlation coefficients for log10 transformed dialkyl phosphate metabolite concentrations in nmol/g creatinine (n=784).

	ICC[a]	ICC[b]
Total dialkyl phosphates[c]	0.26	0.52
Dimethyl alkyl phosphates[e]	0.24	0.48
Diethyl alkyl phosphates[d]	0.22	0.45

a. ICC = Intraclass Correlation Coefficients calculated using a single-measurement, absolute-agreement, and 2-way mixed-effects model.
b. ICC = Intraclass Correlation Coefficients calculated using a mean of three measurements, absolute-agreement, and 2-way mixed-effects model.
c. Total dialkyl phosphates is the sum of DEDTP, DETP, DEP, DMDTP, DMTP and DMP.
d. Diethyl alkyl phosphates is the sum of DEDTP, DETP and DEP.
e. Dimethyl alkyl phosphates is the sum of DMDTP, DMTP and DMP.

Table S2. Associations between dialkyl phosphate metabolite concentrations and maternal thyroid function during pregnancy in TPOAb negative women.

	Mean TSH		Mean FT4		Mean TT4	
<18 weeks gestation	β (95% CI)	*p*	β (95% CI)	*p*	β (95% CI)	*p*
Total dialkyl phosphates						
Model 1	0.00 (-0.04 to 0.04)	0.99	0.30 (-0.36 to 0.96)	0.37	-2.40 (-9.25 to 4.45)	0.49
Model 2	0.00 (-0.04 to 0.04)	0.95	0.36 (-0.29 to 1.02)	0.27	-1.56 (-8.29 to 5.17)	0.65
Dimethyl alkyl phosphates						
Model 1	0.00 (-0.03 to 0.04)	0.86	0.17 (-0.46 to 0.79)	0.60	-2.47 (-8.97 to 4.02)	0.45
Model 2	0.00 (-0.04 to 0.04)	0.90	0.25 (-0.37 to 0.87)	0.43	-2.01 (-8.39 to 4.37)	0.54
Diethyl alkyl phosphates						
Model 1	-0.02 (-0.05 to 0.01)	0.29	0.32 (-0.16 to 0.8)	0.19	-0.18 (-5.15 to 4.8)	0.94
Model 2	-0.02 (-0.05 to 0.01)	0.22	0.33 (-0.14 to 0.79)	0.17	0.73 (-4.12 to 5.57)	0.77

All dialkyl phosphate metabolite concentrations (nmol/g creatinine) and TSH values were log transformed.
N= 668 for TSH, n=667 for FT4, and n=670 for TT4.
Model 1 was adjusted for gestational age at blood sampling and creatinine.
Model 2 was additionally adjusted for maternal age, parity, smoking and ethnicity, child sex, and season.

Table S3. Associations between dialkyl phosphate metabolite concentrations and TPO antibody concentrations

		TPOAbs > 60		TPOAbs > 20	
<18 weeks gestation		OR (95%CI)	*p*	OR (95%CI)	*p*
Total dialkyl phosphates					
	Model 1	0.94 (0.35 to 2.51)	0.90	0.95 (0.42 to 2.16)	0.90
	Model 2	0.81 (0.29 to 2.27)	0.69	0.78 (0.33 to 1.86)	0.58
Dimethyl alkyl phosphates					
	Model 1	0.99 (0.39 to 2.51)	0.98	1.07 (0.49 to 2.34)	0.87
	Model 2	0.87 (0.33 to 2.29)	0.77	0.90 (0.40 to 2.04)	0.81
Diethyl alkyl phosphates					
	Model 1	0.96 (0.47 to 1.96)	0.91	0.82 (0.47 to 1.43)	0.48
	Model 2	0.91 (0.44 to 1.89)	0.80	0.78 (0.45 to 1.36)	0.38

Abbrevations: OR: odds ratio; TPOAbs: thyroperoxidase antibodies (n= 720).
Dialkyl phosphate metabolite concentrations (nmol/g creatinine) were log transformed.
Model 1 was adjusted for gestational age at blood sampling.
Model 2 was additionally adjusted for maternal age, parity, smoking and ethnicity, child sex, and season.

Table S4. Associations between dialkyl phosphate metabolite concentrations during pregnancy per urine collection phase and cord blood thyroid hormone concentrations

		Mean TSH		Mean FT4	
Per urine collection phase		β (95% CI)	*p*	β (95% CI)	*p*
Total DAP < 18 weeks gestation					
	Model 1	-0.05 (-0.12 to 0.02)	0.20	-0.39 (-1.47 to 0.70)	0.48
	Model 2	-0.03 (-0.10 to 0.04)	0.36	-0.33 (-1.43 to 0.77)	0.56
Total DAP 18-25 weeks gestation					
	Model 1	-0.05 (-0.12 to 0.03)	0.20	0.27 (-0.90 to 1.44)	0.65
	Model 2	-0.05 (-0.13 to 0.02)	0.16	0.40 (-0.79 to 1.59)	0.51
Total DAP >25 weeks gestation					
	Model 1	-0.02 (-0.10 to 0.05)	0.54	0.27 (-0.86 to 1.40)	0.64
	Model 2	-0.01 (-0.09 to 0.06)	0.71	0.32 (-0.84 to 1.47)	0.59

All dialkyl phosphate metabolite concentrations (nmol/g creatinine) and TSH values were log transformed.
N= 472 for TSH and n=477 for FT4.
Model 1 was adjusted for gestational age at blood sampling and creatinine.
Model 2 was additionally adjusted for maternal age, parity, smoking and ethnicity, child sex, and season.

Table S5. Variables used to calculate inverse probability of attrition weights.

Variables	Explored	Included
Maternal educational level	x	x
Maternal ethnicity	x	
Maternal age	x	x
Maternal parity	x	x
Maternal alcohol use during pregnancy	x	
Maternal tobacco use during pregnancy	x	
Maternal body mass index	x	x
Household income during pregnancy	x	x
Marital status during pregnancy	x	
Child's birth weight	x	
Gestational age at birth	x	x

Table S6. Associations between dialkyl phosphate metabolite concentrations and maternal thyroid function during pregnancy additionally adjusted for BMI, UICr, TPOAbs, fruit intake, or standardized weights.

	Mean TSH		Mean FT4		Mean TT4	
<18 weeks gestation	β (95% CI)	*p*	β (95% CI)	*p*	β (95% CI)	*p*
Total dialkyl phosphates						
Model 1	0.00 (-0.04 to 0.04)	0.98	0.28 (-0.35 to 0.91)	0.38	-0.78 (-7.37 to 5.80)	0.82
Model 2	0.00 (-0.04 to 0.04)	0.98	0.33 (-0.32 to 0.97)	0.32	-1.89 (-8.58 to 4.80)	0.58
Model 3	0.00 (-0.04 to 0.04)	0.94	0.36 (-0.28 to 0.99)	0.27	-1.16 (-7.75 to 5.43)	0.73
Model 4	-0.01 (-0.05 to 0.03)	0.73	0.34 (-0.30 to 0.99)	0.29	-1.67 (-8.35 to 5.01)	0.62
Model 5	0.00 (-0.04 to 0.04)	0.94	0.31 (-0.32 to 0.94)	0.34	-1.69 (-8.30 to 4.93)	0.62
Dimethyl alkyl phosphates						
Model 1	0.00 (-0.03 to 0.04)	0.82	0.19 (-0.4 to 0.79)	0.53	-1.61 (-7.86 to 4.65)	0.61
Model 2	0.00 (-0.04 to 0.04)	0.83	0.22 (-0.39 to 0.83)	0.48	-2.66 (-9.03 to 3.71)	0.41
Model 3	0.00 (-0.04 to 0.04)	0.89	0.25 (-0.35 to 0.85)	0.41	-1.88 (-8.14 to 4.38)	0.56
Model 4	0.00 (-0.04 to 0.04)	0.91	0.24 (-0.37 to 0.85)	0.44	-2.37 (-8.71 to 3.97)	0.46
Model 5	0.00 (-0.04 to 0.04)	0.87	0.21 (-0.39 to 0.81)	0.50	-2.41 (-8.69 to 3.87)	0.45
Diethyl alkyl phosphates						
Model 1	-0.01 (-0.04 to 0.02)	0.39	0.19 (-0.27 to 0.64)	0.41	1.22 (-3.56 to 5.99)	0.62
Model 2	-0.02 (-0.05 to 0.01)	0.31	0.26 (-0.20 to 0.71)	0.27	0.58 (-4.20 to 5.37)	0.81
Model 3	-0.02 (-0.05 to 0.01)	0.29	0.27 (-0.19 to 0.72)	0.25	0.69 (-4.09 to 5.46)	0.78
Model 4	-0.02 (-0.05 to 0.01)	0.22	0.26 (-0.20 to 0.72)	0.27	0.51 (-4.30 to 5.32)	0.84
Model 5	-0.02 (-0.04 to 0.01)	0.31	0.24 (-0.21 to 0.69)	0.30	0.53 (-4.23 to 5.29)	0.83

All dialkyl phosphate metabolite concentrations (nmol/g creatinine) and TSH values were log transformed.
N= 710 for TSH, n=709 for FT4, and n=712 for TT4.
Model 1 was adjusted for all covariates indicated in model 2 (Table 2), and additionally adjusted for BMI.
Model 2 was adjusted for all covariates in model 2 (Table 2), and additionally adjusted for UICr.
Model 3 was adjusted for all covariates in model 2 (Table 2), and additionally adjusted for TPOAbs.
Model 4 was adjusted for all covariates in model 2 (Table 2), and additionally adjusted for fruit intake.
Model 5 was adjusted for all covariates in model 2 (Table 2), and additionally adjusted for standardized weights.

Table S7. Associations between dialkyl phosphate metabolite concentrations during pregnancy and cord blood thyroid hormone concentrations additionally adjusted for BMI, UICr, TPOAbs, fruit intake, or standardized weights.

		Mean TSH		Mean FT4	
Averaged across pregnancy		β (95% CI)	*p*	β (95% CI)	*p*
Total dialkyl phosphates					
	Model 1	-0.08 (-0.19 to 0.02)	0.11	0.21 (-1.44 to 1.85)	0.80
	Model 2	-0.08 (-0.18 to 0.02)	0.13	0.16 (-1.49 to 1.80)	0.85
	Model 3	-0.07 (-0.18 to 0.03)	0.15	0.27 (-1.35 to 1.90)	0.74
	Model 4	-0.06 (-0.17 to 0.04)	0.23	0.22 (-1.44 to 1.88)	0.80
	Model 5	-0.08 (-0.18 to 0.02)	0.14	0.28 (-1.34 to 1.90)	0.73
Dimethyl alkyl phosphates					
	Model 1	-0.08 (-0.18 to 0.02)	0.10	0.10 (-1.46 to 1.67)	0.90
	Model 2	-0.08 (-0.18 to 0.02)	0.11	0.04 (-1.53 to 1.61)	0.96
	Model 3	-0.07 (-0.17 to 0.02)	0.14	0.16 (-1.39 to 1.71)	0.84
	Model 4	-0.06 (-0.16 to 0.04)	0.21	0.10 (-1.48 to 1.68)	0.90
	Model 5	-0.08 (-0.17 to 0.02)	0.12	0.17 (-1.37 to 1.72)	0.82
Diethyl alkyl phosphates					
	Model 1	-0.04 (-0.12 to 0.04)	0.31	0.34 (-0.97 to 1.65)	0.61
	Model 2	-0.04 (-0.12 to 0.05)	0.39	0.35 (-0.94 to 1.64)	0.60
	Model 3	-0.03 (-0.11 to 0.05)	0.43	0.40 (-0.89 to 1.69)	0.54
	Model 4	-0.03 (-0.11 to 0.06)	0.53	0.37 (-0.94 to 1.67)	0.58
	Model 5	-0.03 (-0.11 to 0.05)	0.42	0.36 (-0.93 to 1.64)	0.58

Average dialkyl phosphate metabolite concentrations (nmol/g creatinine) were computed by the geometric mean of the three urine collection phases.
All dialkyl phosphate metabolite concentrations and TSH values were log transformed.
N= 472 for TSH and n=477 for FT4.
Model 1 was adjusted for all covariates indicated in model 2 (Table 3), and additionally adjusted for BMI.
Model 2 was adjusted for all covariates in model 2 (Table 3), and additionally adjusted for UICr.
Model 3 was adjusted for all covariates in model 2 (Table 3), and additionally adjusted for TPOAbs.
Model 4 was adjusted for all covariates in model 2 (Table 3), and additionally adjusted for fruit intake.
Model 5 was adjusted for all covariates in model 2 (Table 3), and additionally adjusted for standardized weights.

Table S8. Associations between dialkyl phosphate metabolite concentrations (nmol/L) and maternal thyroid function during pregnancy.

	Mean TSH		Mean FT4		Mean TT4	
<18 weeks gestation	β (95% CI)	*p*	β (95% CI)	*p*	β (95% CI)	*p*
Total dialkyl phosphates						
Model 1	-0.02 (-0.05 to 0.01)	0.16	0.23 (-0.26 to 0.72)	0.35	1.93 (-3.20 to 7.06)	0.46
Model 2	-0.02 (-0.05 to 0.01)	0.20	0.27 (-0.21 to 0.75)	0.27	1.49 (-3.53 to 6.50)	0.56
Dimethyl alkyl phosphates						
Model 1	-0.02 (-0.05 to 0.01)	0.24	0.16 (-0.31 to 0.63)	0.51	1.44 (-3.50 to 6.39)	0.57
Model 2	-0.02 (-0.05 to 0.01)	0.29	0.21 (-0.25 to 0.67)	0.37	0.85 (-4.00 to 5.70)	0.73
Diethyl alkyl phosphates						
Model 1	-0.02 (-0.05 to 0.00)	0.06	0.23 (-0.17 to 0.63)	0.26	2.13 (-2.06 to 6.32)	0.32
Model 2	-0.02 (-0.05 to 0.00)	0.06	0.23 (-0.16 to 0.62)	0.24	2.07 (-2.00 to 6.14)	0.32

All dialkyl phosphate metabolite concentrations (nmol/L) and TSH values were log transformed.
N= 710 for TSH, n=709 for FT4, and n=712 for TT4.
Model 1 was adjusted for gestational age at blood sampling.
Model 2 was additionally adjusted for maternal age, parity, smoking and ethnicity, child sex, and season.

Table S9. Associations between dialkyl phosphate metabolite concentrations (nmol/L) during pregnancy and cord blood thyroid hormone concentrations

	Mean TSH		Mean FT4	
Averaged during pregnancy	β (95% CI)	*p*	β (95% CI)	*p*
Average total dialkyl phosphates				
Model 1	-0.04 (-0.11 to 0.04)	0.38	-0.47 (-1.69 to 0.76)	0.45
Model 2	-0.01 (-0.09 to 0.07)	0.77	-0.57 (-1.83 to 0.68)	0.37
Average dimethyl alkyl phosphates				
Model 1	-0.04 (-0.11 to 0.04)	0.34	-0.47 (-1.65 to 0.71)	0.43
Model 2	-0.01 (-0.09 to 0.06)	0.71	-0.59 (-1.80 to 0.63)	0.34
Average diethyl alkyl phosphates				
Model 1	-0.01 (-0.08 to 0.06)	0.86	-0.19 (-1.26 to 0.87)	0.72
Model 2	0.00 (-0.07 to 0.07)	0.97	-0.26 (-1.34 to 0.82)	0.63

Average dialkyl phosphate metabolite concentrations (nmol/L) were computed by the geometric mean of the three urine collection phases.
All dialkyl phosphate metabolite concentrations and TSH values were log transformed.
N= 472 for TSH and n=477 for FT4.
Model 1 was adjusted for gestational age at blood sampling.
Model 2 was additionally adjusted for maternal age, parity, smoking and ethnicity, and child sex.

Table S10. Non-response analysis based on organophosphates data availability.

	Within women with thyroid function data					Within newborns with thyroid function data				
	Available (n=715)		Not available (n=5123)			Available (n=482)		Not available (n=4418)		
Characteristic	Mdn	(95% range)	Mdn	(95% range)	*P*[b]	Mdn	(95% range)	Mdn	(95% range)	*P*[b]
TSH, mU/L	1.31	(0-4.26)	1.34	(0.05-4.55)	0.39	9.52	(2.75-36.92)	9.04	(3.38-32)	0.20
FT4, pmol/L	14.6	(10.3-21.9)	14.8	(10.3-22.4)	0.41	20.9	(15.6-31.8)	20.5	(15.4-28.3)	0.08
TPOAb positivity[a]	42	5.9	263	5.1	0.46	-	-	-	-	-
Gestational age at blood sampling, weeks	12.9	(9.8-17.1)	13.4	(9.8-17.6)	<0.01	40.3	(37.1-42.3)	40.1	(36.6-42.3)	0.40
Urinary iodine to creatinine ratio, µg/g										
Urine collection phase 1	206.7	(70.3-562.1)	210.8	(64.2-615.8)	0.29	203.3	(73.6-538.7)	197.1	(60.9-536.4)	0.43
Urine collection phase 2	229.5	(75.2-528.0)	213.8	(74.6-653.3)	0.29	229.1	(75.2-514.8)	216.3	(70.7-622.6)	0.57
Maternal age, years	31	(20.4-38.9)	30.2	(19.4-38.8)	<0.01	30.9	(20.2-38.7)	30.2	(19.3-38.9)	<0.01
Maternal BMI, Kg/m2	23.1	(18.7-35.6)	23.6	(18.5-35.7)	0.01	23	(18.3-34.6)	23.8	(18.6-35.9)	<0.01
Parity[a]					0.02					0.03
0	441	62	2875	56.7		295	61.5	2418	55.3	
1	188	26.4	1549	30.5		130	27.1	1330	30.4	
≥2	82	11.5	651	12.8		55	11.5	628	14.4	
Smoking[a]					<0.01					0.02
No smoking during pregnancy	501	76.4	3219	71.2		340	76.6	2837	72.6	
Until pregnancy recognized	62	9.5	425	9.4		43	9.7	329	8.4	
Continued during pregnancy	93	14.2	880	19.5		61	13.7	744	19	
Ethnicity[a]					0.01					<0.01
Dutch	410	57.3	2543	52.0		283	58.7	2187	51.8	
Morrocan	38	5.3	295	6.0		25	5.2	295	7.0	
Turkish	40	5.6	414	8.5		26	5.4	381	9.0	
Surinamese	63	8.8	422	8.6		43	8.9	366	8.7	
Other Western	92	12.9	584	11.9		61	12.7	470	11.1	
Other non-western	72	10.1	633	12.9		44	9.1	527	12.5	
Energy adjusted fruit intake, g/d	159.6	(13.9-433.8)	145	(-1.2-429)	<0.01	153.4	(11.5-438.3)	144.2	(-1.8-432.4)	0.13
Child sex[a] (girls %)	353	49.4	2471	49.4	>0.99	234	48.5	2173	49.2	0.82

Data are shown before multiple imputation (see methods section).

a Data shown as n (%).

b P-value for differences calculated using Chi-square test for categorical variables and Kruskall-Wallis H test for continuous variables.

8

Chapter 8

Prenatal exposure to organophosphate pesticides and brain morphology and white matter microstructure in preadolescents

van den Dries, M. A., Lamballais, S., El Marroun, H., Pronk, A., Spaan, S., Ferguson, K. K., Longnecker, M. P., Tiemeier, H., & Guxens, M. (2020).

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Abstract

Background: Prenatal exposure to organophosphate (OP) pesticides associate with impaired neurodevelopment in humans and animal models. However, much uncertainty exists about the brain structural alterations underlying these associations. The objective of this study was to determine whether maternal OP pesticide metabolite concentrations in urine repeatedly measured during gestation are associated with brain morphology and white matter microstructure in 518 preadolescents aged 9–12 years.

Method: Data came from 518 mother–child pairs participating in the Generation R Study, a population-based birth cohort from Rotterdam, the Netherlands. Maternal urine concentrations were determined for 6 dialkylphosphates (DAPs) including 3 dimethyl (DM) and 3 diethyl (DE) alkyl phosphate metabolites, collected at early, mid, and late pregnancy. At child's age 9–12 years, magnetic resonance imaging was performed to obtain T1-weighted images for brain volumes and surface-based cortical thickness and cortical surface area, and diffusion tensor imaging was used to measure white matter microstructure through fractional anisotropy (FA) and mean diffusivity (MD). Linear regression models were fit for the averaged prenatal exposure across pregnancy.

Results: DM and DE metabolite concentrations were not associated with brain volumes, cortical thickness, and cortical surface area. However, a 10-fold increase in averaged DM metabolite concentrations across pregnancy was associated with lower FA (B = −1.00, 95%CI = −1.80, −0.20) and higher MD (B = 0.13, 95%CI = 0.04, 0.21). Similar associations were observed for DE concentrations.

Conclusions: This study provides the first evidence that OP pesticides may alter normal white matter microstructure in children, which could have consequences for normal neurodevelopment. No associations were observed with structural brain morphology, including brain volumes, cortical thickness, and cortical surface area.

Introduction

Organophosphate (OP) pesticides are chemical agents often used in agriculture to protect crops against insects. At present, five billion pounds of pesticides are being applied worldwide and approximately 33% are OP pesticides.[1] Similarly, between 1998 and 2008 one third of the insecticides used in the Netherlands were OP pesticides.[2] In the past decade, the use of OP pesticides has been declining in both the Netherlands and the European Union (EU) due to stricter legislations. However, several OP pesticides such as malathion are currently approved by the EU and OP pesticide residues are frequently detected on tested vegetables and fruits coming from importation.[3,4]

Since OP pesticide residues may persist on or in food after crop harvesting,[5] there is an increasing concern about their potential harmful health effects. The exposure to OP pesticides generally occurs through the consumption of food.[6] However, residential exposure can also occur through the use of insecticides in and around the house or by living in close proximity to agricultural lands were OP pesticides are being applied.[7-12]

It is well established that the exposure to high concentrations of OP pesticides is neurotoxic to both humans and animals.[5,13] However, evidence exist that OP pesticide exposure at fairly low-dose levels may also have a negative health effect.[14,15] OP pesticides are able to pass the placental and the blood-brain barrier[16] and, during gestation, the development of the human brain is especially susceptible to neurotoxic effects.[17] Therefore, pregnancy exposure to low-dose levels of OP pesticides might affect fetal normal brain development.

Although several epidemiological studies have reported associations between pregnancy OP pesticide exposure and offspring's neuropsychological development,[18] much uncertainty exists about the brain structural alterations underlying these associations. Magnetic resonance imaging (MRI) is a useful instrument for addressing these knowledge gaps and can help identify the associations between neurotoxic exposures and brain development.[19] In humans, altered brain morphology and white matter microstructure is associated with impaired cognition, behavior problems, and neurodevelopmental disorders.[20-23] So far, only few animal studies and one small epidemiological study have investigated the effect of OP pesticide exposure on morphological brain measures. Experimental animal studies showed that OP pesticide exposure was associated with smaller brain volumes, both thinning and thickening of the cortex, and alterations of white matter microstructure.[24-26] In humans, prenatal exposure to the OP pesticide chlorpyrifos measured in cord blood was associated with thinner cortices and alterations in cortical surface area in 40 children at 6-11 years of age.[27] However, this previous human study only analyzed a specific OP pesticide, was restricted to a small sample size, and was unable to investigate the exposure across the entire pregnancy. Moreover, no previous epidemiological study investigated

the association between prenatal OP pesticide exposure and white matter microstructure, which has been observed in a previous animal study.[24]

Therefore, the objective of this study was to determine whether maternal OP pesticide metabolite concentrations in urine repeatedly measured during gestation are associated with brain morphology and white matter microstructure in 518 preadolescents at 9–12 years of age. Understanding the association between prenatal OP pesticide exposure and brain morphology and white matter microstructure may help explain the association between pregnancy OP pesticide exposure and offspring's neuropsychological development observed in previous studies. Further, findings of the present study may assist in future policies regarding the regulation of OP pesticide application.

Materials and methods

Study population and follow-up

This research was embedded in the Generation R Study, a population-based cohort from early fetal life onwards in Rotterdam, the Netherlands, which has been described in detail previously.[28] Figure S1 presents a flowchart of this study. Briefly, all pregnant women who lived in the study area in Rotterdam, the Netherlands and were expected to have a delivery between 2002 and 2006 were eligible. A total of 8879 women were enrolled during pregnancy. A random sample of 800 mother-child pairs were selected for assessment of OP pesticide metabolites among the 1449 that provided three spot urine samples during pregnancy and had child's neurodevelopmental data at postnatal visits. Of those, 518 children were included in the present study as they had good quality data on MRI measurements at 9–12 years of age. Human subjects review for the procedure of this study was carried out and approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam (IRB Registration no.: IRB00001482, MEC-2012-165, MEC-2007-413, MEC, 217.595/2002/202, and MEC 198.782.2001.31). Written informed consent for the children and mothers was provided by the mothers.

Urine collection and analysis of OP pesticide metabolites

A more detailed description of urine specimen collection and measurement of OP pesticide metabolites have been published previously[29] and can be found in the supplement (Methods S1). Briefly, 6 non-specific urinary dialkylphosphate (DAP) metabolites of OP pesticides were measured from urine samples collected at <18, 18–25, and >25 weeks of gestation by gas chromatography coupled with tandem mass spectrometry (GC–MS/MS). These include 3 dimethyl alkyl phosphate (DM) and 3 diethyl alkyl phosphate (DE) metabolites. Creatinine concentrations were also measured in order to correct for urinary dilution. All urine samples had detectable concentrations of most metabolites. The intraclass correlation

of DAP metabolite concentrations was weak for a single concentration (0.22–0.26) and moderate for the average of the 3 concentrations (0.51–0.54).[30]

Magnetic resonance imaging

Details of the neuroimaging acquisition and processing can be found in the supplemental material (Methods S2). The global brain metrics derived from T1-weighted images included total brain volume, cerebral and cerebellar white and grey matter volume, and subcortical grey matter volume. Additionally, we focused on the corpus callosum and the subcortical regions: amygdala, caudate nucleus, hippocampus, pallidum, putamen, nucleus accumbens and the thalamus.[31] Surface-based thickness and surface area maps were made of the cerebral cortex.[31] Diffusion tensor imaging (DTI) was used to fit diffusion tensors at each voxel and fractional anisotropy (FA) and mean diffusivity (MD) were computed.[32] Twelve major white matter tracts were identified via probalistic tractography with the FSL plugin AutoPtx.[33,34] These included the forceps minor and major, and the bilateral tracts of the cingulum bundle, corticospinal tract, the inferior and superior longitudinal fasciculi, and the uncinate fasciculus. The mean FA and MD per tract, weighted by the connectivity distribution, were then computed. A confirmatory factor analysis was performed to model a single latent FA and MD measure across the 12 tracts, which represented global FA and MD across the brain.[35] Global FA indicates the tendency for preferential water diffusion in white matter tracts. A lower FA score indicates in general that the comprising axons are less densely packed and the directionality of the water diffusion is not uniformly directed as compared with well-organized tracts. Global MD describes the magnitude of average water diffusion in all directions within brain tissue, with higher values generally occurring in white matter tracts that show a less well-organized structure.[36,37]

Potential confounders

Potential adjustment variables were selected a priori defined as the minimal sufficient adjustment set with a Directed Acyclic Graph (DAG) using the Dagitty software.[38] The DAG was based on previous studies of prenatal OP pesticide exposure and neurodevelopment and on biologically plausible covariate–exposure and covariate–outcome associations observed in our data (see Fig. S2). We further included adjustment variables that are ancestors of the exposure and ancestors of the outcome to increase precision. The adjustment variables were household income [less than 1200 euro/month (i.e., less than the social security level of the Netherlands), 1200–2000 euro/month, more than 2000 euro/month], maternal highest achieved level of education [low (less than 3 years of high school), intermediate (3 or more years of secondary education), and high (university degree or higher vocational training)], maternal ethnical background (Dutch, other Western, and non-Western), maternal age at enrolment, marital status (married/living with partner versus single), maternal parity (0, 1, or 2 or more children), maternal smoking habits during pregnancy (none, only until pregnancy known, or continued after pregnancy known), maternal

gestational alcohol use [none, only until pregnancy known, continued infrequently (<1 glass/week) or continued regularly (≥1 glass/week)], maternal pre-pregnancy body mass index (BMI) (kg/m2), maternal IQ (assessed when the mother-child pairs visited the research center for the 6-year examination and measured by using the computerized Ravens Advanced Progressive Matrices Test, set I,[39] child sex, and the child age at the MRI scan.

Statistical methods

Total DM (nmol/l) was created by summing dimethylphosphate, dimethylthiophosphate, and dimethyldithiophosphate metabolite concentrations. Total DE (nmol/l) was defined as the sum of diethylphosphate, diethylthiophosphate, and diethyldithiophosphate metabolite concentrations. Total DAP (nmol/l) was created by summing the 6 metabolites. These concentrations were creatinine adjusted (nmol/g creatinine) and transformed using a log transformation (base 10) to improve linearity of the dose-response relation and model fit. Few concentrations were missing because of an inadequate sample or machine error. We therefore imputed missing concentrations (<1%) and missing confounder information 10 times by using the Multivariate Imputation by Chained Equations (MICE) package in R.[40,41] We included total brain volume and global FA in the imputation procedure as predictors, but we did not impute them.

As a first step, we applied linear regression to examine the association of averaged DM, DE, and DAP concentrations over pregnancy with brain volumes. To account for multiple testing (14 tests for each exposure), we applied the false discovery rate (FDR) correction. As a second step, we investigated metabolite concentrations – brain volume associations for each exposure time point (early, mid, and late pregnancy) separately. We performed this second step for the identification of possible periods of susceptibility and to have the ability to compare our findings with studies that only used one urine sample during gestation to measure the exposure to OP pesticides. These analyses were also corrected for multiple testing using the FDR correction. The same multiple linear regressions were applied for the DAP – global white matter tract (FA and MD) associations. Post-hoc analyses were run on the 12 major individual white matter tracts when the analysis yielded a significant association between prenatal DAP metabolite concentrations and global white matter tracts. We explored whole-brain vertex-wise statistics using the QDECR R package (https://qdecr.com) for total DM, DE, and DAP metabolite concentrations in association with local cortical thickness and cortical surface area. Vertex-wise analyses were corrected for multiple testing by the application of Gaussian Monte Carlo null-Z simulations (The cluster-forming threshold defined as $p < 0.001$). Next, these analyses were also corrected by applying a Bonferroni adjustment for the analyses of both hemispheres.

All models were adjusted for potential confounders described above. Additionally, we adjusted models of subcortical and cerebellar volumes for intracranial volume to ascertain

relativity to head size. Models of the other volumes were not adjusted for intracranial volume as they were highly correlated (between r = 0.81 and r = 0.93).

As sensitivity analyses, first, we investigated potential effect modification by sex via interaction terms (P-value for interaction<0.05) to compare our results with previous studies who observed sex specific effects.[27,42] Second, we applied inverse probability weighting to adjust all models for loss to follow-up and to deal with potential selection bias because participants included in this study were older, had higher educational level, and more frequently Dutch as compared to the complete Generation R Study cohort.[29] Third, because diet and the intake of healthy nutrients may confound the association between prenatal OP pesticide exposure (e.g., residues on fruits)[29] and brain development (e.g., healthy nutrients) (Figure S2), we performed a sensitivity analyses in which we additionally adjusted for maternal fruit and vegetables intake. The consumption of fruit and vegetables was assessed in the first trimester using a modified version of a validated food frequency questionnaire and was adjusted for energy intake.[43]

Results

Descriptive analysis

The median age of the mothers at enrolment was 31.2 years (IQR = 5.4) and the median age of the child at MRI assessment was 9.8 years (IQR = 0.3) (Table 1). The majority of mothers participating in this study were ethnically Dutch (61.4%), were nulliparous (66.3%), were none smokers (79.1%), had a high educational level (60.2%), and had a high income (73.6%). Total DAP metabolite concentrations comprised mostly DM metabolite concentrations (Table 2). The median nmol/g creatinine concentrations were comparable across the three sampling periods. The median total brain volume was 1215 cm3 (IQR = 138 cm3) and median FA was 0.0 (IQR = 2.3) (Table S1).

OP pesticide metabolite concentrations and brain volume

No associations were observed between averaged maternal DM and DE metabolite concentrations and all brain volumes (Table 3). When specific pregnancy periods were analyzed separately, higher DM and DE metabolite concentrations at >25 weeks of gestation were associated with lower thalamus volume, higher DM metabolite concentration at 18–25 weeks of gestation was associated with higher putamen volume, and higher DE metabolite concentrations at >25 weeks of gestation were associated with lower cerebellum cortex volume (Table S2). However, these associations did not remain after correction for multiple testing. The results for the total DAP metabolite concentrations were similar to the results observed for the DM and DE metabolite concentrations (Table S3).

Table 1. Demographic and lifestyle characteristics of 518 mother-child pairs from the Generation R Study population.

		Median (25th, 75th percentile) or %
Maternal characteristics		
Age		31.2 (28.6, 34.0)
	Missing, *n*	-
Ethnicity		
	Dutch	61.4%
	Other western	13.1%
	Non-western	25.5%
	Missing, *n*	-
Educational level		
	Low	11.2%
	Intermediate	28.6%
	High	60.2%
	Missing, *n*	11
Household income		
	<1200	11.6%
	1200-2000	14.8%
	>2000	73.6%
	Missing, *n*	52
Non-verbal IQ		100.0 (90.0, 107.0)
	Missing, *n*	7
Body mass index		23.0 (21.2, 25.9)
	Missing, *n*	2
Parity		
	0	66.3%
	1	24.2%
	>1	9.5%
	Missing, *n*	2
Smoking during pregnancy		
	No smoking during pregnancy	79.1%
	Until pregnancy recognized	8.8%
	Continued during pregnancy	12.1%
	Missing, *n*	40
Alcohol consumption during pregnancy		
	No consumption during pregnancy	33.2%
	Until pregnancy recognized	17.7%
	Continued occasionally	42.1%
	Continued frequently	7.0%
	Missing, *n*	21
Child characteristics		
Child age at assessment		9.8 (9.6, 9.9)
	Missing, *n*	-
Child sex		
	Male	49.4%
	Female	50.6%
	Missing, *n*	-

Table 2. Descriptive statistics of pregnancy DAP metabolite concentrations (n=518).

	nmol/g creatinine					nmol/l				
	min	**p25**	**p50**	**p75**	**max**	**min**	**p25**	**p50**	**p75**	**max**
DM metabolites in nmol/g creatinine [a]										
< 18 weeks	6.6	153.6	255.3	420.4	6106.5	0.9	96.1	183.4	346.7	2627.3
18 – 25 weeks	24.8	184.2	272.1	433.6	2444.1	7.6	99.6	190.8	336.4	2396.8
> 25 weeks	29.2	165.8	248.9	397.6	2857.8	10.5	103.9	194.2	326.6	3300.5
Averaged	26.3	191.3	269.3	361.8	1381.0	14.7	118.5	179.6	289.4	1105.1
DE metabolites in nmol/g creatinine [b]										
< 18 weeks	0.0	25.3	46.4	86.3	3030.5	0.0	16.0	31.3	66.2	6818.6
18 – 25 weeks	3.3	25.2	43.4	79.6	624.3	0.6	13.9	30.1	58.4	1093.4
> 25 weeks	4.1	22.0	43.9	81.5	671.4	1.1	14.7	31.5	64.4	538.2
Averaged	2.6	29.4	44.3	68.9	601.4	3.2	19.3	30.7	49.8	407.3
DAP metabolites in nmol/g creatinine [c]										
< 18 weeks	15.4	197.3	321.4	521.7	6444.5	6.3	119.6	224.7	422.2	7798.7
18 – 25 weeks	41.0	222.2	323.0	519.8	2817.0	10.0	123.2	235.9	406.1	3056.7
> 25 weeks	42.1	204.1	308.0	495.7	3003.1	15.2	127.2	228.8	403.4	3332.6
Averaged	36.7	234.6	329.9	441.2	1818.8	30.2	144.4	220.7	348.9	1259.3

Abbreviations: Min=minimum, p25=25th percentile, P50=median (50th percentile), p75=75th percentile, max=maximum, DM= Dimethyl alkyl phosphates, DE= Diethyl alkyl phosphates, DAP= Total dialkyl phosphates.
a. DM is the sum of dimethylphosphate (DMP), dimethylthiophosphate (DMTP), and dimethyldithiophosphate (DMDTP).
b. DE is the sum of diethylphosphate (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP).
c. DAP is the sum of DMDTP, DMTP, DMP, DEDTP, DETP, and DEP.

OP pesticide metabolite concentrations and white matter microstructure

Table 3 presents the association between averaged DM and DE metabolite concentrations and white matter microstructure. We observed an association between a 10-fold increase in averaged DM and DE metabolite concentrations and lower FA [B = −1.00 (95%CI: −1.80, −0.20) and B = −0.63 (95%CI: −1.24, −0.02), respectively]. Next, a 10-fold increase in averaged DM and DE metabolite concentrations were associated with higher MD [B = 0.13 (95%CI: 0.04, 0.21) and B = 0.06 (95%CI: 0.00, 0.13), respectively]. Regarding the specific pregnancy periods, we observed similar associations for DM and DE concentrations at <18 weeks and at 18–25 weeks of gestation (Table S3). The associations between maternal DAP metabolite concentrations and white matter microstructure were comparable to the results of DM and DE metabolite concentrations (Table S3).

Regarding the individual 12 major white matter tracts, we observed that DM metabolite concentrations averaged across pregnancy were associated with lower FA and higher

Table 3. Adjusted [a] association between averaged log10 transformed maternal concentrations of DM [b] and DE [c] metabolite concentrations in nmol/g creatinine and brain volumes (n=441) and white matter microstructure (n=474) assessed at child age 10 years.

	Averaged DM metabolite concentrations in nmol/g creatinine		Averaged DE metabolite concentrations in nmol/g creatinine	
	B	95%CI	B	95%CI
Brain volumes				
Total brain	12.81	-26.31 to 51.92	0.22	-28.98 to 29.42
Total gray	4.69	-18.75 to 28.13	-3.38	-20.88 to 14.12
Subcortical gray matter	0.26	-1.53 to 2.04	-0.69	-2.03 to 0.65
Cerebral white matter	8.16	-9.57 to 25.90	4.27	-8.98 to 17.53
Thalamus [d]	-0.36	-0.74 to 0.02	-0.18	-0.47 to 0.11
Caudate [d]	0.18	-0.18 to 0.55	-0.11	-0.38 to 0.16
Putamen [d]	0.42	0.00 to 0.85	-0.01	-0.33 to 0.31
Pallidum [d]	0.05	-0.10 to 0.20	-0.05	-0.16 to 0.06
Hippocampus [d]	-0.10	-0.35 to 0.16	0.02	-0.17 to 0.21
Amygdala [d]	0.05	-0.08 to 0.18	0.05	-0.05 to 0.15
Nucleus accumbens [d]	0.00	-0.07 to 0.07	0.00	-0.05 to 0.06
Cerebellum cortex [d]	-2.68	-6.51 to 1.15	-2.30	-5.16 to 0.57
Cerebellar white matter [d]	-0.08	-1.08 to 0.91	-0.46	-1.20 to 0.28
Corpus callosum [d]	-0.06	-0.27 to 0.16	-0.01	-0.17 to 0.15
White matter microstructure				
Global FA	-1.00	-1.80 to -0.20	-0.63	-1.24 to -0.02
Global MD	0.13	0.04 to 0.21	0.06	0.00 to 0.13

Abbreviations: DM= Dimethyl alkyl phosphates, DE= Diethyl alkyl phosphates, FA= fractional anisotropy, MD= mean diffusivity

a. Adjusted for age of child during the magnetic resonance imaging assessment and sex of child, and maternal age, ethnicity (Dutch, other-western, and non-western), education (low, intermediate, and high), income (low, middle, and high), marital status (married/living with partner and single), alcohol consumption during pregnancy (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasionally alcohol consumption during pregnancy, and frequently alcohol consumption during pregnancy), body mass index, parity (0, 1, and 2+), and smoking during pregnancy (no smoking during pregnancy, smoked until pregnancy was known, and smoked during pregnancy).

b. DM is the sum of dimethylphosphate, dimethylthiophosphate, and dimethyldithiophosphate.

c. DE is the sum of diethylphosphate, diethylthiophosphate, and diethyldithiophosphate.

d. Additionally adjusted for intracranial volume.

MD in most of the tracts except for the uncinate fasciculus tract of left hemisphere, the forceps major, and the corticospinal tract of the right hemisphere (Fig. 1 and Table S4). We observed that higher averaged DE metabolite concentrations were associated with lower FA in the superior longitudinal fasciculus tract of the left hemisphere and the corticospinal tracts, and with higher MD in the cingulate gyrus of the cingulum tract of the left hemisphere, the forceps minor tract, and the inferior longitudinal fasciculus tract of the left hemisphere (Fig. 1 and Table S5). The results of the total DAP metabolite

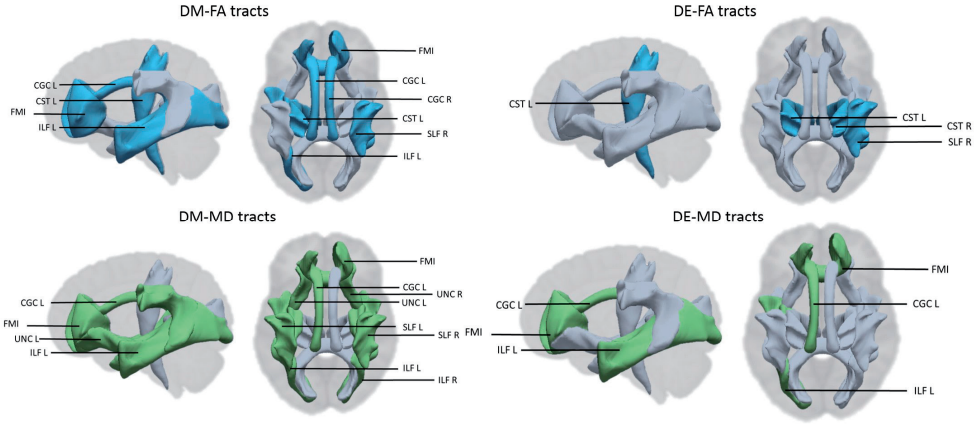

Figure 1. The white matter tracts of global fractional anisotropy (FA) and global mean diffusivity (MD) that are associated (blue= negative, green = positive) with <18 weeks, 18-25 weeks and averaged maternal dimethyl (DM) and diethyl (DE) alkyl phosphate metabolite concentrations in nmol/g creatinine. CGC= Cingulum bundle, CST= corticospinal tract, ILF= inferior longitudinal fasciculus, SLF= superior longitudinal fasciculus, UNC=uncinated fasciculus, FMI= forceps minor, L=left hemisphere, R= right hemisphere.

concentrations were also almost identical to the results observed for the DM and DE metabolite concentrations (Table S6).

OP pesticide metabolite concentrations and cortical thickness and cortical surface area

We did not find any evidence of an association between prenatal DM, DE, or DAP metabolite concentrations and cortical thickness and cortical surface area using whole-brain vertex-wise analyses (data not shown).

Sensitivity analyses

Effect modification by sex was not observed in the association between averaged DAP metabolite concentrations with cortical and subcortical volumes or with white matter microstructure (Table S7). When models were re-run correcting for the potential selection bias using inverse probability weighting, results were similar to the main analyses (Table S8). Of note, a 10-fold increase in averaged DAP metabolite concentrations was significantly associated with a 0.50 (95%CI = −0.89, −0.11) decrease in thalamus volume, but the result did not survive the multiple testing correction. Finally, the results in which we additionally adjusted for maternal diet were similar to the main results (Table S9).

Discussion

In this population-based study, we observed that OP pesticide metabolite concentrations measured during pregnancy were not associated with brain morphological measures including brain volumes, cortical thickness, and cortical surface area in pre-adolescents at 9–12 years of age. However, we showed that higher prenatal exposure to OP pesticides, in particular during early- and mid-pregnancy, was associated with lower FA and higher MD, generally considered as indicators for atypical white matter microstructure. When we explored the specific white matter tracts, we observed that OP pesticide exposure was associated with projection, association, limbic system, and callosal fibers.

Although prior studies have noted the importance of the use of neuroimaging tools to address existing research gaps by identifying structural neurotoxic effects of prenatal OP pesticide exposure on the brain,[19] only one small epidemiological study has investigated this research question.[27] This study observed that prenatal exposure to chlorpyrifos, which devolves into the DE metabolites diethylphosphate and diethylthiophosphate,[44] was predictive of enlargement of the cortical surface in several areas including superior and middle temporal gyrus, post-central gyrus, superior frontal gyrus, cuneus and precuneus, and gyrus rectus.[27] Furthermore, increased exposure was associated with lower cortical thickness of frontal, temporal, and parietal regions. In contrast to these findings, no evidence of an association with cortical surface area and cortical thickness was observed in our study. The inconsistency in the results may be explained by the differences in OP pesticide exposure, in exposure assessment methodology, or in study populations. Rauh et al. (2012) measured a single OP pesticide chlorpyrifos in cord blood, while in our study we measured DAP metabolites at early-, mid-, and late-pregnancy in urine as a biomarker of OP pesticide exposure. DAP metabolites provide non-specific data about the total exposure to several OP pesticides instead of the exposure to a single OP pesticide. Mothers in the current study were most likely exposed to a combination of different OP pesticides that also produce DE metabolites. Of all the insecticides that were applied in 2004 in the Netherlands, 32% were OP pesticides that produce DM metabolites and only 1% were pesticides that generate DE metabolites.[2] Of the latter, the OP pesticide chlorpyrifos accounted for 1/3 of the total generated DE metabolites. This may suggest that exposure to chlorpyrifos may have been lower in our population. However, between 2004 and 2006 OP pesticide residues of chlorpyrifos coming from importation have been detected on tested vegetables and fruits.[3] Of note, DAP metabolite concentrations in this study are higher compared to most other birth-cohort studies.[29] Other differences between the studies relate to socio-economic status, as the population in the previous study was socially disadvantaged. It is conceivable that in these populations unmeasured background risk factors related to both chlorpyrifos exposure and brain morphology might lead to potential residual confounding.

To our knowledge, this is the first epidemiological study that investigated prenatal exposure to OP pesticides and white matter microstructure. In preadolescents aged 9–12 years, the development of many white matter tracts, such as projection of the prefrontal cortex, is still ongoing.[45] Altered maturation of white matter microstructure might therefore result in neurodevelopmental problems with long-term clinical implications. Studies have found that altered white matter microstructure is associated with impaired cognition, behavior problems, and neurodevelopmental disorders.[20] We observed that increased OP pesticide exposure during pregnancy was associated with lower FA and higher MD of white matter and that the direction of the associations was consistent across most specific tracts. To help interpret these results we calculated the association of child age with white matter microstructure, as age is a robust determinant of the latter. A one-year increase in age was associated with 0.88 (95%CI = 0.35, 1.42) increase in global FA and a 0.10 (95%CI = −0.16, −0.05) decrease in global MD. This implies that, for example, a 10-fold increase in averaged DM concentrations during pregnancy has a similar effect as being 1.1 years younger in terms of white matter microstructure.

Global FA and MD are indicators of white matter microstructure.[36] FA describes the propensity for enhanced water diffusion in the white matter tracts whereas MD expresses the scale of average water diffusion in every direction within brain tissue.[36] A lower FA and higher MD can be a result of several reasons including lower packing of axons, higher membrane permeability, disturbance of internal axonal structure, and decreased myelination.[45] Animal studies also observed similar associations in white matter microstructure in relationship with exposure to OP pesticides. Prenatally chlorpyrifos exposed guinea pigs had lower FA and higher MD within the corpus callosum and the amygdala and rats postnatal (day 1 until day 6) exposed to chlorpyrifos had a decreased expression of the myelin-associated glycoprotein in the brain which is crucial for the preservation of the mature myelinated unit.[24,46] Moreover, chlorpyrifos exposure reduces the polymerization of tubulin.[47] Tubulin is an protein which plays an important role in the creation of microtubules which are needed for the preservation of the structural and functional integrity of axons.[47,48] In our study we further found an association between OP pesticide exposure and white matter microstructure specific tracts present in projection, association, limbic system, and callosal fibers. Further work is required to confirm our observed association between prenatal OP pesticide exposure and altered white matter microstructure in children.

We observed that the first and second trimester (<18 weeks, and 18–25 weeks) OP pesticide exposure were driving the association with lower FA and higher MD. White matter growth starts in early gestation and myelination begins in the second trimester.[17,49,50] OP pesticide exposure has been shown to disrupt the expression of genes and proteins important for the myelination.[51] During the second trimester, the development of white

matter is especially dependable on signaling pathways such as extracellular ligands, secreted molecules, and transcriptional regulations.[52] Thus, OP pesticide exposure may alter the courses of later brain development by influencing axonal growth adhering and group formation and white matter myelination via gene expression alteration.

This study has several limitations. First, urinary DAP metabolite concentrations provide information regarding the joint exposure to multiple OP pesticides instead of providing specific information regarding the exact OP pesticide exposure.[53-55] It is therefore unknown to which specific OP parent pesticide(s) our study population was exposed. However, the use of DAP metabolites as biomarkers of OP pesticides is also a strength because it allows for the identification and comparison of OP pesticide exposure levels within and between study populations.[56] Second, DAP metabolites are characterized by a short half-life and are excreted in urine within one or two days. This implies that the biomarker concentrations may differ from day-to-day within each subject as a consequence of variable contact with exposure sources (e.g., variable diet patterns) and result in (within-subject) temporal variability.[6,57-59] Although we included 3 urine spot samples which is more frequent than most other studies exploring the association between prenatal OP pesticide exposure and neurodevelopment, it would be preferable to collect more urine specimens during pregnancy to reduce the measurement error and attenuation bias caused by the within-subject variability.[60] Third, this study was restricted by the nonappearance of information on possible residential pesticide use by the participant, another household member, or a professional exterminator. Participants in this study might have been exposed through the use of residential products which may contain OP pesticides such as insecticides for the lawn and garden (e.g., emulsifiable concentrate), insecticides for house plants, residential pest products (e.g., fly control insecticides and moth killer cassettes), and flea products for pets. Although the use of products that contain OP pesticides is unlikely to confound the association between prenatal OP pesticide exposure and brain morphology, such information would be helpful in determining the exact sources of the exposure. The Generation R Study is representative of an urban population of which the exposure to OP pesticides most likely occurs through diet.[29] The results of this study may therefore not be fully generalizable to semi-urban and rural areas in the Netherlands where the source of OP pesticide exposure could be different. Finally, this study was limited by the absence of information on exposure to other types of pesticides. While we included many possible confounders in our analyses, we cannot eliminate the existence of potential residual confounding in this study as a consequence of unidentified background risk factors that are predictive of OP pesticide exposure and brain development.

The present study has a number of strengths. This study has a large sample size and the availability of many potential confounders such as the IQ of the mother and socio-economic

factors. Further, the scanning procedure in which all brains were scanned using the same MRI scanner and software to reduce potential measurement error is another strength.

In conclusion, prenatal OP pesticide exposure was not associated with brain volumes, cortical thickness, and cortical surface area in preadolescents aged 9–12 years. However, we found that prenatal OP pesticide exposure was associated with lower FA and higher MD of white matter, and that early and mid-pregnancy exposure were driving these associations. These findings suggest that prenatal exposure to worldwide commonly applied OP pesticides may alter normal white matter microstructure development in children, which could have consequences for normal neurodevelopment. Besides structural brain changes, functional brain alteration may also provide opportunities to deepen the understanding of the effects of prenatal exposure to OP pesticides on the brain, as a recent study has done.[42] Future studies on brain imaging are warranted to reproduce our findings, as well as to investigate the mediating role of the structural and functional brain alterations in the association between prenatal exposure to OP pesticides and neuropsychological development. If the findings of this study are confirmed, public health policies that aim towards stricter regulation and control of OP pesticides application should be implemented both in Europe and worldwide.

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Supplemental Material

Method S1

Maternal spot urine specimens were collected during early, middle, and late pregnancy (<18, 18-25, >25 weeks of gestational age, respectively). Details of urine specimen collection have been described elsewhere.[1] Briefly, all urine samples were collected between 8 am and 8 pm in 100 mL polypropylene urine collection containers that were kept for a maximum of 20 h in a cold room (4°C) before being frozen at −20°C in 20 mL portions in 25 mL polypropylene vials.

Measurements of 6 non-specific urinary dialkylphosphate (DAP) metabolites of OP pesticides were conducted at the Institut National de Santé Publique (INSPQ) in Quebec, Canada, using gas chromatography coupled with tandem mass spectrometry (GC–MS/MS).[2] More details of DAP metabolite measurements can be found elsewhere.[3] Briefly, 3 dimethyl alkyl phosphates (DM) metabolites (dimethylphosphate (DMP), dimethylthiophosphate (DMTP), and dimethyldithiophosphate (DMDTP)) and 3 diethyl alkyl phosphates (DE) metabolites (diethylphosphate (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP)) were measured. The limit of detection (LOD) was 0.26 µg/l for DMP, 0.40 for DMTP, 0.09 for DMDTP, 0.50 for DEP, 0.12 for DETP, and 0.06 for DEDTP. The inter-day precision of the method during this project, expressed as the coefficient of variation (CV) and measured with the inclusion of the values <LOD, varied between 4.2–8.8% for DEDTP, 4.1–7.2% for DEP, 5.0–9.1% for DETP, 5.5–7.1% for DMDTP, 5.3–8.0% for DMP, and 5.5–7.7% for DMTP based on reference materials (clinical check-urine level II 637 E-495 and MRM E-459).[4]

Apart for DEDTP, only a small proportion of the concentrations were below the limit of detection (LOD).[4] The lab reported concentrations below the LOD (DMP = 0.26 µg/L, DMTP = 0.40 µg/L, DMDTP = 0.09 µg/L, DEP = 0.50 µg/L, DETP = 0.12 µg/L, and DEDTP = 0.06 µg/L) were included in the data analysis. Molar concentrations were used to facilitate comparison of our results with those from other studies. To account for urinary dilution, creatinine concentrations were determined based on the Jaffe reaction and molar concentrations were converted to nmol/g creatinine.[5]

The intraclass correlation (ICC) – estimated by using a 2-way mixed-effects model with absolute-agreement – for DAP metabolite concentrations varied between 0.22 and 0.26 for a single measurement and between 0.51 and 0.54 for the mean of the three measurements.[6]

Method S2

Neuroimaging was performed using a 3T General Electric scanner (Discovery MR750W; GE Worldwide, Milwaukee, WI) with an 8-channel head coil. The protocol has been described elsewhere.[7] T1-weighted data were collected using an inversion recovery fast spoiled gradient recalled sequence (TR = 8.77 ms, TE = 3.4 ms, TI = 600 ms, flip angle = 10° , field of view = 220 mm x 220 mm, acquisition matrix = 220 x 220, ARC acceleration factor = 2, number of slices = 230, slice thickness = 1.0 mm). Diffusion-weighted images were collected with 3 b = 0 volumes and 35 noncollineair diffusion directions using an echo planar imaging sequence (TR = 12,500 ms, TE = 72 ms, field of view = 240 mm x 240 mm, acquisition matrix = 120 x 120, Asset acceleration factor = 2, number of slices = 65, slice thickness = 2 mm, b = 900 s/mm2).

Cortical reconstruction and volumetric segmentation were carried out with FreeSurfer Image Analysis Suite 6.0.[8,9] Non-brain tissue was removed and images were normalized for B1 field inhomogeneities, followed by tissue segmentation, as well as parcellation in accordance with the Desikan-Killiany atlas.[10] Global metrics included total brain volume, cerebral and cerebellar white and gray matter volume, and subcortical gray matter volume. Furthermore, we included volumes of the corpus callosum, the thalamus, caudate nucleus, putamen, pallidum, hippocampus, amygdala, and nucleus accumbens. Finally, surface-based thickness and surface area maps were made of the cerebral cortex. The maps were coregistered to a standard stereotaxic space for all participants and consequently smoothed with a 10 mm full-width half-maximum Gaussian kernel. All images were inspected for surface reconstruction accuracy using automated and manual methods.[9]

Diffusion tensor imaging (DTI) images were further processed with the FMRIB Software Library (FSL), version 5.0.9.[11] Non-brain tissue was removed, and images were corrected for eddy-current artifacts and translations/rotations due to motion. The RESTORE method from the Camino toolkit was then used to fit diffusion tensors at each voxel,[12] and fractional anisotropy (FA) and mean diffusivity (MD) were computed. Twelve major white matter tracts (cingulum bundle, corticospinal tract, forceps major, forceps minor, inferior longitudinal fasciculus, superior longitudinal fasciculus and the uncinate fasciculus) were identified via probalistic tractography with the FSL plugin AutoPtx.[13,14] The mean FA and MD per tract, weighted by the connectivity distribution, were then computed. A confirmatory factor analysis was performed to model a single latent FA and MD measures across the 12 tracts, which represented global FA and MD across the brain.[15]

Table S1. Descriptive statistics of brain volume (n=441) and white matter microstructure (n=474) measures assessed by magnetic resonance imaging at child age 10 years.

	min	25th percentile	Median	75th percentile	max
Brain volumes					
Total brain	857.4	1140.7	1214.5	1278.4	1592.2
Total gray volume	546.5	721.8	768.2	801.5	970.6
Subcortical gray matter	46.0	57.3	59.9	63.2	76.4
Cerebral white matter	290.6	391.9	420.3	450.1	592.4
Thalamus	10.9	14.0	14.7	15.7	20.2
Caudate	5.6	7.5	8.2	8.8	11.3
Putamen	6.5	10.0	10.8	11.5	13.6
Pallidum	2.8	3.6	3.9	4.1	5.1
Hippocampus	5.9	7.5	7.9	8.5	10.1
Amygdala	2.3	3.3	3.5	3.7	4.6
Nucleus accumbens	0.7	1.2	1.4	1.5	1.9
Cerebellum cortex	89.5	111.2	117.7	124.9	156.3
Cerebellar white matter	18.1	24.0	25.8	27.5	35.3
Corpus callosum	2.2	3.0	3.3	3.6	5.2
White matter microstructure					
Global FA	-6.0	-1.3	0.0	1.0	4.7
Global MD	-0.6	-0.1	0.0	0.1	0.8

Abbreviations: min= minimum, max=maximum, FA= fractional anisotropy, MD=mean diffusivity.

Table S2. Adjusted[a] association between log10 transformed maternal concentrations of DM[b] and DE[c] metabolite concentrations in nmol/g creatinine and brain volumes (n=441) and white matter microstructure (n=474) assessed at child age 10 years.

		<18 weeks		18-25 weeks		>25 weeks	
Brain volumes		**B**	**95%CI**	**B**	**95%CI**	**B**	**95%CI**
Total brain	DMs	-1.98	-26.29 to 22.34	10.76	-17.38 to 38.90	10.67	-15.99 to 37.33
	DEs	5.67	-14.50 to 25.85	-0.85	-22.94 to 21.25	-3.93	-25.94 to 18.09
Total gray volume	DMs	-1.24	-15.75 to 13.26	5.11	-11.75 to 21.97	3.49	-12.48 to 19.45
	DEs	2.53	-9.55 to 14.61	-5.32	-18.52 to 7.88	-3.20	-16.38 to 9.97
Subcortical gray matter	DMs	-0.42	-1.52 to 0.69	0.74	-0.55 to 2.03	0.20	-1.02 to 1.42
	DEs	-0.17	-1.09 to 0.76	-0.11	-1.13 to 0.91	-0.69	-1.69 to 0.31
Cerebral white matter	DMs	-0.70	-11.79 to 10.39	5.76	-6.99 to 18.51	7.11	-4.99 to 19.20
	DEs	3.31	-5.86 to 12.48	4.87	-5.18 to 14.92	-0.07	-10.07 to 9.94
Thalamus[d]	DMs	-0.11	-0.35 to 0.13	-0.10	-0.38 to 0.18	-0.28	-0.54 to -0.02
	DEs	0.00	-0.20 to 0.20	-0.10	-0.32 to 0.12	-0.23	-0.45 to -0.02
Caudate[d]	DMs	0.05	-0.18 to 0.28	0.19	-0.07 to 0.45	0.02	-0.23 to 0.27
	DEs	-0.07	-0.26 to 0.12	-0.14	-0.35 to 0.07	0.02	-0.19 to 0.23
Putamen[d]	DMs	0.07	-0.19 to 0.34	0.35	0.04 to 0.66	0.19	-0.10 to 0.48
	DEs	0.03	-0.19 to 0.25	0.05	-0.19 to 0.29	-0.07	-0.31 to 0.16
Pallidum[d]	DMs	0.00	-0.09 to 0.09	0.09	-0.02 to 0.20	-0.01	-0.11 to 0.09
	DEs	-0.03	-0.11 to 0.05	0.02	-0.06 to 0.11	-0.05	-0.14 to 0.03
Hippocampus[d]	DMs	-0.12	-0.28 to 0.04	0.07	-0.12 to 0.25	-0.04	-0.22 to 0.13
	DEs	0.05	-0.09 to 0.18	0.01	-0.13 to 0.16	-0.03	-0.17 to 0.12
Amygdala[d]	DMs	-0.03	-0.12 to 0.05	0.08	-0.01 to 0.18	0.03	-0.06 to 0.13
	DEs	0.05	-0.02 to 0.12	0.03	-0.04 to 0.11	0.01	-0.07 to 0.08
Nucleus accumbens[d]	DMs	-0.01	-0.06 to 0.03	0.04	-0.01 to 0.09	-0.02	-0.07 to 0.03
	DEs	-0.01	-0.05 to 0.03	0.01	-0.03 to 0.05	0.02	-0.03 to 0.06
Cerebellum cortex[d]	DMs	-0.54	-2.93 to 1.84	-2.42	-5.17 to 0.32	-0.92	-3.53 to 1.70
	DEs	-0.72	-2.69 to 1.25	-0.99	-3.17 to 1.19	-2.31	-4.45 to -0.16
Cerebellar white matter[d]	DMs	0.05	-0.56 to 0.67	-0.08	-0.79 to 0.64	-0.11	-0.79 to 0.57
	DEs	-0.09	-0.60 to 0.42	-0.35	-0.91 to 0.22	-0.46	-1.02 to 0.10
Corpus callosum[d]	DMs	0.01	-0.12 to 0.14	-0.03	-0.18 to 0.12	-0.06	-0.21 to 0.08
	DEs	0.01	-0.10 to 0.12	0.04	-0.08 to 0.16	-0.07	-0.19 to 0.04
White matter microstructure		**B**	**95%CI**	**B**	**95%CI**	**B**	**95%CI**
Global FA	DMs	-0.60	-1.10 to -0.11	-0.62	-1.17 to -0.06	-0.05	-0.60 to 0.49
	DEs	-0.46	-0.89 to -0.03	-0.25	-0.68 to 0.19	-0.22	-0.68 to 0.23
Global MD	DMs	0.04	-0.01 to 0.09	0.10	0.04 to 0.16	0.03	-0.03 to 0.08
	DEs	0.04	0.00 to 0.09	0.03	-0.02 to 0.07	0.02	-0.02 to 0.07

Abbreviations: DM= Dimethyl alkyl phosphates, DE= Diethyl alkyl phosphates

a. Adjusted for age of child during the magnetic resonance imaging assessment, sex of child, and maternal age, ethnicity (Dutch, other-western, and non-western), education (low, intermediate, and high), income (low, middle, and high), marital status (married/living with partner and single), alcohol consumption during pregnancy (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasionally alcohol consumption during pregnancy, and frequently alcohol consumption during pregnancy), body mass index, parity (0, 1, and 2+), and smoking during pregnancy (no smoking during pregnancy, smoked until pregnancy was known, and smoked during pregnancy).

b. DM is the sum of dimethylphosphate, dimethylthiophosphate, and dimethyldithiophosphate.

c. DE is the sum of diethylphosphate, diethylthiophosphate, and diethyldithiophosphate.

d. Additionally adjusted for intracranial volume.

Table S3. Adjusted[a] association between averaged log10 transformed maternal concentrations of DAP[b] metabolite concentrations in nmol/g creatinine and brain volumes (n=441) and white matter microstructure (n=474) assessed at child age 10 years.

	Averaged		<18 weeks		18-25 weeks		>25 weeks	
	B	95%CI	B	95%CI	B	95%CI	B	95%CI
Brain volumes								
Total brain	10.37	-29.32 to 50.05	-3.22	-28.28 to 21.84	11.75	-17.47 to 40.96	8.70	-19.11 to 36.52
Total gray volume	2.54	-21.23 to 26.31	-2.16	-17.11 to 12.79	4.36	-13.14 to 21.87	2.50	-14.15 to 19.16
Subcortical gray matter	-0.01	-1.82 to 1.80	-0.52	-1.66 to 0.62	0.72	-0.62 to 2.07	-0.03	-1.30 to 1.25
Cerebral white matter	8.04	-9.97 to 26.05	-0.90	-12.34 to 10.54	7.49	-5.75 to 20.73	6.23	-6.40 to 18.85
Thalamus[c]	-0.38	-0.77 to 0.01	-0.12	-0.37 to 0.12	-0.10	-0.39 to 0.19	-0.32	-0.59 to -0.05
Caudate[c]	0.05	-0.32 to 0.43	-0.01	-0.24 to 0.23	0.09	-0.18 to 0.37	0.00	-0.26 to 0.26
Putamen[c]	0.40	-0.03 to 0.83	0.09	-0.19 to 0.36	0.35	0.03 to 0.67	0.17	-0.14 to 0.47
Pallidum[c]	0.03	-0.13 to 0.18	-0.01	-0.11 to 0.09	0.09	-0.02 to 0.20	-0.03	-0.13 to 0.08
Hippocampus[c]	-0.06	-0.32 to 0.20	-0.08	-0.24 to 0.09	0.07	-0.12 to 0.26	-0.05	-0.23 to 0.13
Amygdala[c]	0.06	-0.08 to 0.19	-0.01	-0.10 to 0.07	0.08	-0.02 to 0.18	0.03	-0.06 to 0.12
Nucleus accumbens[c]	0.00	-0.07 to 0.08	-0.02	-0.06 to 0.03	0.05	-0.01 to 0.10	-0.02	-0.07 to 0.04
Cerebellum cortex[c]	-2.96	-6.85 to 0.92	-0.71	-3.17 to 1.74	-2.38	-5.24 to 0.48	-1.34	-4.07 to 1.38
Cerebellar white matter[c]	-0.16	-1.17 to 0.84	-0.01	-0.65 to 0.63	-0.09	-0.84 to 0.65	-0.15	-0.85 to 0.56
Corpus callosum[c]	-0.06	-0.28 to 0.15	0.00	-0.14 to 0.14	-0.01	-0.17 to 0.15	-0.08	-0.23 to 0.07
White matter microstructure								
Global FA	-1.00	-1.82 to -0.17	-0.68	-1.21 to -0.16	-0.62	-1.20 to -0.04	-0.01	-0.59 to 0.56
Global MD	0.13	0.05 to 0.22	0.05	0.00 to 0.11	0.10	0.04 to 0.16	0.03	-0.03 to 0.09

Abbreviations: DAP total dialkyl phosphate, FA= fractional anisotropy, MD= mean diffusivity

a. Adjusted for age of child during the magnetic resonance imaging assessment, sex of child, and maternal age, ethnicity (Dutch, other-western, and non-western), education (low, intermediate, and high), income (low, middle, and high), marital status (married/living with partner and single), alcohol consumption during pregnancy (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasionally alcohol consumption during pregnancy, and frequently alcohol consumption during pregnancy), body mass index, parity (0, 1, and 2+), and smoking during pregnancy (no smoking during pregnancy, smoked until pregnancy was known, and smoked during pregnancy).

b. DAP is the sum of dimethylphosphate, dimethylthiophosphate, dimethyldithiophosphate, diethylphosphate, diethylthiophosphate, and diethyldithiophosphate.

c. Additionally adjusted for intracranial volume.

Table S4. The adjusted[a] association between log10 transformed maternal DM[b] metabolite concentrations in nmol/g creatinine and fractional anisotropy and mean diffusivity of twelve white matter tracts (n=474).

	<18 weeks		18-25 weeks		Averaged	
	B	95% CI	B	95% CI	B	95% CI
Fractional anisotropy						
Uncinate fasciculus L	0.001	-0.008 to 0.009	-0.002	-0.011 to 0.007	-0.002	-0.015 to 0.012
Uncinate fasciculus R	-0.003	-0.010 to 0.004	-0.003	-0.011 to 0.005	-0.007	-0.018 to 0.005
Cingulate gyrus part of cingulum L	-0.013	-0.025 to -0.001	-0.003	-0.016 to 0.011	-0.013	-0.033 to 0.006
Cingulate gyrus part of cingulum R	-0.016	-0.027 to -0.005	-0.008	-0.021 to 0.005	-0.019	-0.037 to -0.001
Superior longitudinal fasciculus L	-0.004	-0.010 to 0.002	-0.003	-0.010 to 0.004	-0.004	-0.014 to 0.006
Superior longitudinal fasciculus R	-0.007	-0.014 to -0.001	-0.010	-0.018 to -0.003	-0.013	-0.024 to -0.003
Forceps minor	-0.009	-0.018 to 0.001	-0.004	-0.014 to 0.007	-0.017	-0.032 to -0.002
Forceps major	-0.003	-0.012 to 0.007	-0.007	-0.018 to 0.003	-0.004	-0.019 to 0.012
Inferior longitudinal fasciculus L	-0.006	-0.012 to 0.000	-0.008	-0.015 to -0.001	-0.008	-0.018 to 0.002
Inferior longitudinal fasciculus R	-0.004	-0.010 to 0.002	-0.007	-0.014 to 0.000	-0.008	-0.018 to 0.002
Corticospinal tract L	-0.006	-0.011 to 0.000	-0.002	-0.008 to 0.004	-0.009	-0.018 to 0.000
Corticospinal tract R	-0.003	-0.009 to 0.002	-0.003	-0.009 to 0.004	-0.008	-0.017 to 0.001
Mean diffusivity[c]						
Uncinate fasciculus L	0.001	-0.005 to 0.007	0.008	0.002 to 0.015	0.008	-0.002 to 0.018
Uncinate fasciculus R	0.005	-0.001 to 0.011	0.008	0.002 to 0.015	0.015	0.006 to 0.025
Cingulate gyrus part of cingulum L	0.008	0.000 to 0.016	0.014	0.004 to 0.023	0.019	0.005 to 0.032
Cingulate gyrus part of cingulum R	0.007	-0.001 to 0.015	0.005	-0.004 to 0.014	0.010	-0.003 to 0.023
Superior longitudinal fasciculus L	0.005	-0.001 to 0.011	0.011	0.004 to 0.018	0.014	0.004 to 0.024
Superior longitudinal fasciculus R	0.008	0.001 to 0.015	0.012	0.004 to 0.020	0.015	0.003 to 0.027
Forceps minor	0.000	-0.009 to 0.009	0.016	0.006 to 0.026	0.017	0.003 to 0.032
Forceps major	-0.006	-0.024 to 0.013	0.015	-0.006 to 0.036	0.010	-0.020 to 0.041
Inferior longitudinal fasciculus L	0.002	-0.006 to 0.009	0.013	0.004 to 0.021	0.012	0.000 to 0.025
Inferior longitudinal fasciculus R	0.005	-0.004 to 0.013	0.012	0.003 to 0.022	0.016	0.002 to 0.030
Corticospinal tract L	0.003	-0.003 to 0.010	0.003	-0.004 to 0.010	0.007	-0.003 to 0.017
Corticospinal tract R	0.001	-0.004 to 0.006	0.005	-0.001 to 0.011	0.004	-0.004 to 0.012

Abbreviations: DM= dimethyl alkyl phosphates, L= left hemisphere, R= right hemisphere.

a. Adjusted for age of child during the magnetic resonance imaging assessment, sex of child, and maternal age, ethnicity (Dutch, other-western, and non-western), education (low, intermediate, and high), income (low, middle, and high), marital status (married/living with partner and single), alcohol consumption during pregnancy (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasionally alcohol consumption during pregnancy, and frequently alcohol consumption during pregnancy), body mass index, parity (0, 1, and 2+), and smoking during pregnancy (no smoking during pregnancy, smoked until pregnancy was known, and smoked during pregnancy).

b. DM is the sum of dimethylphosphate, dimethylthiophosphate, and dimethyldithiophosphate.

c. Mean diffusivity values were multiplied by 10^9.

Table S5. The adjusted[a] association between log10 transformed maternal DE[b] metabolite concentrations in nmol/g creatinine and fractional anisotropy and mean diffusivity of twelve white matter tracts (n=474).

	<18 weeks			18-25 weeks			Averaged		
	B	95% CI		B	95% CI		B	95% CI	
Fractional anisotropy									
Uncinate fasciculus L	0.000	-0.006	0.006	0.002	-0.005	0.009	0.001	-0.009	0.011
Uncinate fasciculus R	-0.003	-0.008	0.002	0.000	-0.006	0.006	-0.005	-0.013	0.004
Cingulate gyrus part of cingulum L	-0.008	-0.017	0.001	-0.003	-0.013	0.008	-0.010	-0.025	0.005
Cingulate gyrus part of cingulum R	-0.006	-0.014	0.003	0.000	-0.009	0.010	-0.006	-0.019	0.008
Superior longitudinal fasciculus L	-0.003	-0.007	0.002	0.000	-0.006	0.005	-0.001	-0.009	0.006
Superior longitudinal fasciculus R	-0.004	-0.009	0.001	-0.004	-0.010	0.001	-0.009	-0.017	-0.001
Forceps minor	-0.004	-0.011	0.003	-0.005	-0.013	0.003	-0.010	-0.022	0.001
Forceps major	-0.002	-0.009	0.005	0.000	-0.008	0.008	0.001	-0.010	0.013
Inferior longitudinal fasciculus L	-0.002	-0.006	0.003	-0.001	-0.006	0.004	-0.005	-0.012	0.003
Inferior longitudinal fasciculus R	-0.001	-0.005	0.004	-0.001	-0.007	0.004	-0.003	-0.011	0.005
Corticospinal tract L	-0.006	-0.010	-0.002	-0.005	-0.009	0.000	-0.011	-0.018	-0.004
Corticospinal tract R	-0.005	-0.009	-0.001	-0.004	-0.009	0.000	-0.010	-0.017	-0.004
Mean diffusivity[c]									
Uncinate fasciculus L	-0.001	-0.005	0.004	0.001	-0.004	0.006	0.002	-0.005	0.009
Uncinate fasciculus R	0.003	-0.002	0.007	0.001	-0.004	0.006	0.006	-0.002	0.013
Cingulate gyrus part of cingulum L	0.006	0.000	0.012	0.008	0.001	0.015	0.012	0.002	0.023
Cingulate gyrus part of cingulum R	0.004	-0.002	0.010	0.002	-0.005	0.009	0.005	-0.005	0.015
Superior longitudinal fasciculus L	0.003	-0.002	0.008	0.002	-0.004	0.007	0.006	-0.002	0.014
Superior longitudinal fasciculus R	0.003	-0.003	0.008	0.001	-0.005	0.007	0.004	-0.005	0.013
Forceps minor	0.005	-0.002	0.011	0.010	0.002	0.017	0.016	0.005	0.027
Forceps major	-0.005	-0.019	0.009	0.002	-0.014	0.018	-0.005	-0.028	0.018
Inferior longitudinal fasciculus L	0.004	-0.002	0.009	0.006	-0.001	0.012	0.010	0.001	0.019
Inferior longitudinal fasciculus R	0.002	-0.004	0.009	-0.001	-0.008	0.006	0.002	-0.009	0.012
Corticospinal tract L	0.003	-0.002	0.007	0.002	-0.003	0.007	0.006	-0.002	0.013
Corticospinal tract R	0.000	-0.003	0.004	0.001	-0.003	0.005	0.002	-0.005	0.008

Abbreviations: DE= diethyl alkyl phosphates, L= left hemisphere, R= right hemisphere.

a. Adjusted for age of child during the magnetic resonance imaging assessment, sex of child, and maternal age, ethnicity (Dutch, other-western, and non-western), education (low, intermediate, and high), income (low, middle, and high), marital status (married/living with partner and single), alcohol consumption during pregnancy (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasionally alcohol consumption during pregnancy, and frequently alcohol consumption during pregnancy), body mass index, parity (0, 1, and 2+), and smoking during pregnancy (no smoking during pregnancy, smoked until pregnancy was known, and smoked during pregnancy).

b. DE is the sum diethylphosphate, diethylthiophosphate, and diethyldithiophosphate.

c. Mean diffusivity values were multiplied by 10^9.

Table S6. The adjusted[a] association between log10 transformed maternal DAP[b] metabolite concentrations in nmol/g creatinine and fractional anisotropy and mean diffusivity of twelve white matter tracts (n=474).

	<18 weeks		18-25 weeks		Averaged	
	B	95% CI	B	95% CI	B	95% CI
Fractional anisotropy						
Uncinate fasciculus L	0.001	-0.008 to 0.010	-0.001	-0.011 to 0.009	-0.002	-0.015 to 0.012
Uncinate fasciculus R	-0.005	-0.012 to 0.003	-0.002	-0.011 to 0.006	-0.008	-0.020 to 0.004
Cingulate gyrus part of cingulum L	-0.015	-0.028 to -0.002	-0.003	-0.017 to 0.011	-0.014	-0.034 to 0.006
Cingulate gyrus part of cingulum R	-0.016	-0.028 to -0.004	-0.006	-0.019 to 0.007	-0.016	-0.035 to 0.003
Superior longitudinal fasciculus L	-0.005	-0.011 to 0.002	-0.004	-0.011 to 0.004	-0.004	-0.014 to 0.007
Superior longitudinal fasciculus R	-0.008	-0.015 to -0.001	-0.011	-0.019 to -0.003	-0.013	-0.024 to -0.002
Forceps minor	-0.010	-0.020 to 0.000	-0.004	-0.015 to 0.006	-0.017	-0.033 to -0.002
Forceps major	-0.002	-0.012 to 0.008	-0.007	-0.018 to 0.004	-0.001	-0.017 to 0.015
Inferior longitudinal fasciculus L	-0.007	-0.013 to 0.000	-0.007	-0.015 to 0.000	-0.008	-0.019 to 0.002
Inferior longitudinal fasciculus R	-0.004	-0.011 to 0.002	-0.006	-0.013 to 0.001	-0.007	-0.017 to 0.004
Corticospinal tract L	-0.006	-0.012 to -0.001	-0.003	-0.009 to 0.004	-0.010	-0.019 to -0.001
Corticospinal tract R	-0.005	-0.010 to 0.001	-0.004	-0.010 to 0.003	-0.010	-0.019 to -0.001
Mean diffusivity[c]						
Uncinate fasciculus L	0.000	-0.006 to 0.007	0.008	0.001 to 0.015	0.008	-0.002 to 0.018
Uncinate fasciculus R	0.006	-0.001 to 0.012	0.008	0.001 to 0.015	0.015	0.005 to 0.025
Cingulate gyrus part of cingulum L	0.010	0.002 to 0.019	0.015	0.006 to 0.025	0.021	0.008 to 0.035
Cingulate gyrus part of cingulum R	0.008	-0.001 to 0.017	0.005	-0.005 to 0.014	0.010	-0.004 to 0.024
Superior longitudinal fasciculus L	0.006	-0.001 to 0.013	0.011	0.003 to 0.018	0.014	0.003 to 0.025
Superior longitudinal fasciculus R	0.009	0.001 to 0.016	0.011	0.003 to 0.020	0.014	0.002 to 0.026
Forceps minor	0.002	-0.007 to 0.012	0.018	0.007 to 0.028	0.021	0.006 to 0.036
Forceps major	-0.008	-0.028 to 0.012	0.014	-0.008 to 0.035	0.007	-0.024 to 0.039
Inferior longitudinal fasciculus L	0.004	-0.004 to 0.012	0.014	0.005 to 0.022	0.015	0.002 to 0.027
Inferior longitudinal fasciculus R	0.006	-0.003 to 0.015	0.010	0.001 to 0.020	0.014	0.000 to 0.028
Corticospinal tract L	0.004	-0.002 to 0.011	0.003	-0.004 to 0.010	0.008	-0.002 to 0.019
Corticospinal tract R	0.001	-0.004 to 0.007	0.005	-0.001 to 0.010	0.004	-0.004 to 0.012

Abbreviations: DAP= Total dialkyl phosphates, L= left hemisphere, R= right hemisphere.

a. Adjusted for age of child during the magnetic resonance imaging assessment, sex of child, and maternal age, ethnicity (Dutch, other-western, and non-western), education (low, intermediate, and high), income (low, middle, and high), marital status (married/living with partner and single), alcohol consumption during pregnancy (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasionally alcohol consumption during pregnancy, and frequently alcohol consumption during pregnancy), body mass index, parity (0, 1, and 2+), and smoking during pregnancy (no smoking during pregnancy, smoked until pregnancy was known, and smoked during pregnancy).

b. DAP is the sum of dimethylphosphate, dimethylthiophosphate, dimethyldithiophosphate, diethylphosphate, diethylthiophosphate, and diethyldithiophosphate.

c. Mean diffusivity values were multiplied by 10^9.

Table S7. P-value of the interaction between averaged log10 transformed DAP[a] metabolite concentrations in nmol/g creatinine and sex in the adjusted[b] association between averaged log10 transformed maternal concentrations of DAP metabolite and brain volumes (n=441) and white matter microstructure (n=474).

Brain volumes	**P-value for interaction**
Total brain	0.052
Total gray	0.065
Subcortical gray matter	0.264
Cerebral white matter	0.072
Thalamus[c]	0.961
Caudate[c]	0.212
Putamen[c]	0.899
Pallidum[c]	0.960
Hippocampus[c]	0.826
Amygdala[c]	0.598
Nucleus accumbens[c]	0.307
Cerebellum cortex[c]	0.757
Cerebellar white matter[c]	0.703
Corpus callosum[c]	0.298
White matter microstructure	**P-value for interaction**
Global FA	0.241
Global MD	0.610

Abbreviations: DAP= Dialkyl phosphates, FA= fractional anisotropy, MD= mean diffusivity
a. DAP is the sum of dimethylphosphate, dimethylthiophosphate, dimethyldithiophosphate, diethylphosphate, diethylthiophosphate, and diethyldithiophosphate.
b. Adjusted for age of child during the magnetic resonance imaging assessment, sex of child, and maternal age, ethnicity (Dutch, other-western, and non-western), education (low, intermediate, and high), income (low, middle, and high), marital status (married/living with partner and single), alcohol consumption during pregnancy (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasionally alcohol consumption during pregnancy, and frequently alcohol consumption during pregnancy), body mass index, parity (0, 1, and 2+), and smoking during pregnancy (no smoking during pregnancy, smoked until pregnancy was known, and smoked during pregnancy).
c. Additionally adjusted for intracranial volume.

Table S8. Adjusted[a] inverse probability weighted association between averaged log10 transformed maternal concentrations of DAP[b] metabolite concentrations in nmol/g creatinine and brain volumes (n=441) and white matter microstructure (n=474) assessed at child age 10 years.

	Averaged DAP metabolite concentrations in nmol/g creatinine			
Brain volumes	**B**	**95%CI**		
Total brain	14.73	-25.37	to	54.84
Total gray	3.39	-20.77	to	27.54
Subcortical gray matter	0.00	-1.84	to	1.84
Cerebral white matter	11.51	-6.53	to	29.55
Thalamus[c]	-0.50	-0.89	to	-0.11
Caudate[c]	0.08	-0.29	to	0.44
Putamen[c]	0.40	-0.03	to	0.83
Pallidum[c]	0.00	-0.15	to	0.15
Hippocampus[c]	-0.08	-0.33	to	0.18
Amygdala[c]	0.08	-0.05	to	0.22
Nucleus accumbens[c]	0.02	-0.06	to	0.09
Cerebellum cortex[c]	-2.54	-6.45	to	1.36
Cerebellar white matter[c]	-0.15	-1.16	to	0.87
Corpus callosum[c]	-0.02	-0.23	to	0.20
White matter microstructure	**B**	**95%CI**		
Global FA	-1.03	-1.86	to	-0.20
Global MD	0.13	0.04	to	0.22

Abbreviations: DAP= Dialkyl phosphates, FA= fractional anisotropy, MD= mean diffusivity

a. Adjusted for age of child during the magnetic resonance imaging assessment, sex of child, and maternal age, ethnicity (Dutch, other-western, and non-western), education (low, intermediate, and high), income (low, middle, and high), marital status (married/living with partner and single), alcohol consumption during pregnancy (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasionally alcohol consumption during pregnancy, and frequently alcohol consumption during pregnancy), body mass index, parity (0, 1, and 2+), and smoking during pregnancy (no smoking during pregnancy, smoked until pregnancy was known, and smoked during pregnancy).

b. DAP is the sum of dimethylphosphate, dimethylthiophosphate, dimethyldithiophosphate, diethylphosphate, diethylthiophosphate, and diethyldithiophosphate.

c. Additionally adjusted for intracranial volume.

Table S9. Adjusted[a] association between averaged log10 transformed maternal concentrations of DAP[b] metabolite concentrations in nmol/g creatinine and brain volumes (n=441) and white matter microstructure (n=474) assessed at child age 10 years with additional adjustment for maternal fruit and vegetable intake.

	Averaged DAP metabolite concentrations in nmol/g creatinine			
Brain volumes	**B**	**95%CI**		
Total brain	11.09	-29.35	to	51.53
Total gray	2.17	-22.00	to	26.35
Subcortical gray matter	0.02	-1.83	to	1.86
Cerebral white matter	9.05	-9.37	to	27.46
Thalamus[c]	-0.35	-0.74	to	0.04
Caudate[c]	0.04	-0.34	to	0.41
Putamen[c]	0.43	-0.01	to	0.86
Pallidum[c]	0.05	-0.10	to	0.21
Hippocampus[c]	-0.08	-0.34	to	0.19
Amygdala[c]	0.05	-0.09	to	0.19
Nucleus accumbens[c]	0.00	-0.07	to	0.08
Cerebellum cortex[c]	-2.58	-6.51	to	1.34
Cerebellar white matter[c]	-0.09	-1.11	to	0.93
Corpus callosum[c]	-0.03	-0.25	to	0.18
White matter microstructure	**B**	**95%CI**		
Global FA	-0.96	-1.81	to	-0.11
Global MD	0.14	0.05	to	0.23

Abbreviations: DAP= Dialkyl phosphates, FA= fractional anisotropy, MD= mean diffusivity
a. Adjusted for age of child during the magnetic resonance imaging assessment, sex of child, and maternal age, ethnicity (Dutch, other-western, and non-western), education (low, intermediate, and high), income (low, middle, and high), marital status (married/living with partner and single), alcohol consumption during pregnancy (no alcohol consumption during pregnancy, alcohol consumption until pregnancy was known, occasionally alcohol consumption during pregnancy, and frequently alcohol consumption during pregnancy), body mass index, parity (0, 1, and 2+), smoking during pregnancy (no smoking during pregnancy, smoked until pregnancy was known, and smoked during pregnancy), energy adjusted maternal fruit intake, and energy adjusted vegetable intake.
b. DAP is the sum of dimethylphosphate, dimethylthiophosphate, dimethyldithiophosphate, diethylphosphate, diethylthiophosphate, and diethyldithiophosphate.
c. Additionally adjusted for intracranial volume.

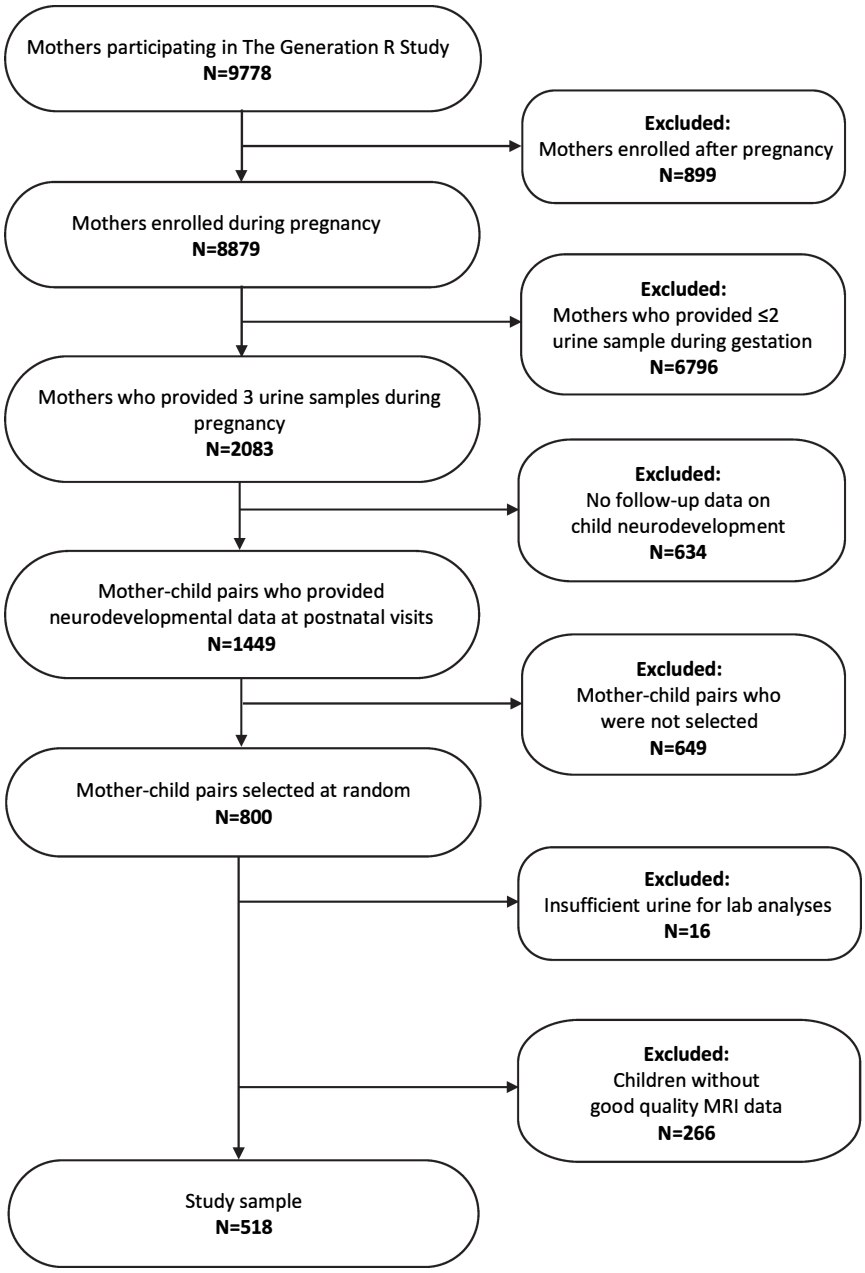

Figure S1. Flowchart of study population.

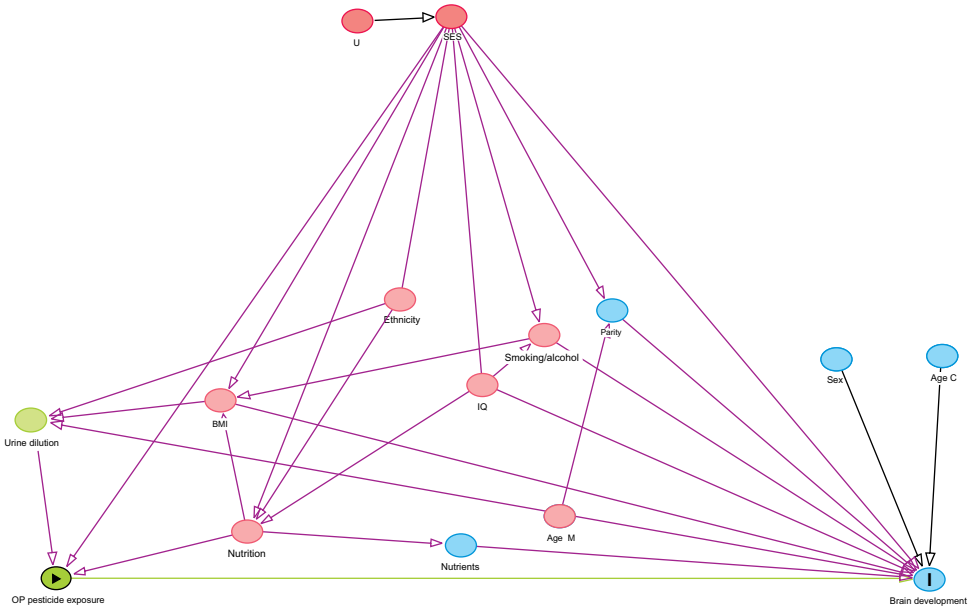

Figure S2. Directed Acyclic Graph of the OP pesticide exposure and brain development. Potential adjustment variables were selected a priori defined with a Directed Acyclic Graph (DAG) using the Dagitty software (Textor et al. 2017). The DAG was based on previous studies of OP pesticide exposure and child neurodevelopment and on biologically plausible covariate–exposure and covariate–outcome associations observed in our data. Green circles represent ancestors of the exposure, blue circles ancestors of the outcome, pink circles ancestors of both exposure and outcome. BMI= Maternal body mass index, SES= socioeconomic status (maternal education, household income and marital status), age C= child age at assessment, age M= age mother, IQ= maternal nonverbal intelligent quotient, U=unobserved ancestor of socioeconomic status.

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9

Chapter 9

Organophosphate pesticide exposure in pregnancy in association with ultrasound and delivery measures of fetal growth

Ferguson, K. K., van den Dries, M. A., Gaillard, R., Pronk, A., Spaan, S., Tiemeier, H., & Jaddoe, V.W.V. (2019).

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Abstract

Background: Perturbations in fetal growth may have adverse consequences for childhood and later life health. Organophosphate pesticide (OP) exposure has been associated with reduced birth weight at delivery but results are not consistent.

Objectives: We investigated this question by utilizing ultrasound measures of size in utero in combination with measures from delivery.

Methods: Within Generation R, a population-based prospective cohort conducted between 2002 and 2006 in Rotterdam, Netherlands, we measured dialkyl phosphates (DAPs), OP metabolites, in urine samples from early, middle, and late pregnancy and created a subject-specific average to estimate OP exposure (n=784). Ultrasound measures of head circumference, femur length, and estimated fetal weight from middle and late pregnancy and delivery measures were converted to standard deviation scores (SDS). Associations with DAP average were examined in linear mixed effects models that included an interaction term between gestational age at measurement and DAP average to investigate whether the relationship differed over time. Windows of vulnerability to exposure were assessed by modeling urinary DAPs from each visit in relation to growth measurements.

Results: A 10-fold increase in average DAPs was associated with a –0.53 SDS decrease in fetal length (95% CI =–0.83, –0.23) and a –0.32 SDS decrease in estimated fetal weight (95% CI =–0.59, –0.04) at 20 weeks of gestation. These differences corresponded to 5% and 6% decreases relative to the mean. Effect estimates were greatest in magnitude for DAP concentrations measured early in pregnancy. Associations between average DAPs and growth measures at delivery were positive but not significant for head circumference and length and were null for weight.

Conclusions: Maternal urinary DAPs were associated with decreased fetal weight and length measured during mid-pregnancy, but not at delivery.

Introduction

Perturbations in normal fetal growth are linked to numerous adverse health outcomes both in childhood[1] and later life.[2] Suboptimal fetal growth is classically approximated by birth weight at delivery with or without adjustment for gestational duration. However, for diagnostic purposes, assessment of fetal growth longitudinally during pregnancy is preferred.[3] In research, repeated ultrasound measures of growth allow for the a) improved ability to detect deviations from normality that occur during gestation, not just at delivery; b) investigation of rates of change in growth, rather than a snapshot of size; and c) assessment of specific fetal growth measures, such as length as an indicator of skeletal size, which are not fully captured by birth weight alone. Utilizing these data, researchers have demonstrated specific time periods in pregnancy where changes in rate of growth may have the greatest impact on childhood health outcomes (e.g., adiposity, neurodevelopment).[4,5] Similarly, studies of environmental factors and fetal growth have used these data to augment understanding of windows when exposures have the strongest influence on growth and which specific anthropometric parameters are most affected (e.g., head circumference vs. weight).[6-9]

To our knowledge, longitudinal ultrasounds in pregnancy have not been used to investigate the association between organophosphate pesticide (OP) exposure and fetal growth. OPs such as dimethoate and parathion are a class of high-production insecticides with neurotoxic capacity. Exposure can occur through occupational use or proximity to areas with agricultural application, but most populations are exposed through diet.[10-13] There is strong biologic plausibility for an effect of OPs on in utero growth and development through interference with adenyl cyclase activity, which is crucial for cell differentiation,[14] disruption of normal thyroid hormone function in the mother or fetus,[15] or dysregulation of nutrient transport across the placenta [16]. Evidence for an association with birth weight has been demonstrated in some but not all rodent studies of OPs.[17-20] Results from human studies on the association between biomarkers of OPs and birth weight, including a recent pooled analysis, have also been ambiguous, and associations may differ by individuals' ability to detoxify OPs by the paraoxonase.[21]

In the present study, we investigated the association between maternal OP exposure in pregnancy and fetal growth as assessed by repeated ultrasound measurements during pregnancy in combination with neonatal assessments. We utilized urinary dialkyl phosphates (DAPs), metabolites of OPs, measured in urine samples collected at three time points in pregnancy as proxies of exposure. Our primary aim was to assess associations of average DAPs over pregnancy with repeated measures of head circumference, length, and weight measured at two time points during pregnancy by ultrasound and at delivery. Our secondary aim was to identify potential windows of vulnerability to exposure by

examining outcomes in association with DAP concentrations at each individual time point. We additionally examined effect modification of these associations by fetal sex and PON1 genotype.

Methods

Study Population

Generation R is a prospective population-based birth cohort designed to identify early environmental and genetic determinants of development throughout life and which has been described in detail previously.[22] Briefly, all mothers who resided in the study area in Rotterdam, Netherlands, and had a delivery date between April 2002 and January 2006 were eligible. Mothers were enrolled during pregnancy or in the first months after the birth of their child when newborns visited the routine child health centers. Among the 9,778 mothers who participated in the study 8,879 (91%) were enrolled during pregnancy. Among the 4,918 women enrolled during pregnancy between February 2004 and January 2006, spot urine specimens during early, middle, and late pregnancy (<18, 18–25, and >25 weeks of gestational age, respectively) were collected at the time of routine ultrasound examinations. In total, 2,083 women provided a complete set of three urine specimens. The study protocol underwent human subjects review at Erasmus Medical Center, Rotterdam, Netherlands (institutional review board registration no. IRB00001482, MEC 198.782.2001.31). Mothers provided written informed consent for themselves and their children.

Among the women with urine specimens collected at each of the three visits in pregnancy, 1,449 had complete information on childhood health assessments.[22] From these women, 800 were randomly selected for a study designed to assess the relationship between prenatal exposure to OPs and childhood neurodevelopmental outcomes.[13] Due to limitations in urine sample volume, 784 individuals were included in the final study population (n=778 with three samples; n=5 with two samples; n=1 with one sample). A flow chart describing the selection process is shown in Figure S1. Women in this subset had higher education levels and were slightly older and a greater proportion were Dutch compared with the broader Generation R cohort.[22]

Ultrasound and delivery measures of size

During pregnancy, ultrasound scans were performed to calculate gestational age and to measure fetal growth on the entire study population, as described in detail elsewhere.[23] Head circumference and length were measured in middle and late pregnancy and estimated fetal weight for each time point was calculated using the formula of Hadlock et al. (1985).[24] At birth, head circumference, length, and weight were measured. Standard

deviation scores (SDS) for each measurement were calculated using longitudinal growth curves that accounted for gestational age at measurement but not fetal sex.[23]

Urinary dialkyl phosphate measurement

At each of the three study visits, urine samples were collected from participants in polypropylene cups and stored until analysis at 20°C.[25,26] Six nonspecific DAPs were measured using gas chromatography coupled with tandem mass spectrometry (GC-MS/MS) at the Institute National de Santé Publique (INSPQ) in Quebec, Canada, with methods described in detail elsewhere.[26,27] These measurements included three dimethyl metabolites (dimethylphosphate, dimethythiophosphate, and dimethyldithiophosphate; DMPs) and three diethyl metabolites (diethylphosphate, diethylthiophosphate, and diethyldithiophosphate; DEPs). Limits of detection were between 0.06 and 0.5μg/L and coefficients of variation for inter-day reliability were <10%.[13] Values below the limit of detection were imputed with machine-reported values when available. We calculated nanomolar sums of DMPs, DEPs, and total DAPs using molecular weights.[13,26] To adjust for urine dilution, we measured creatinine concentrations using the Jaffe reaction and corrected each sum so that final concentrations are presented in nanomoles per gram creatinine. Finally, we calculated subject-specific geometric averages of DMP, DEP, and DAP concentrations from levels measured at each of the three visits in pregnancy in order to create more stable estimates of exposure for our primary aim.[13]

PON1 genotyping

Cord blood from 523 children included in the present analysis was genotyped using Illumina 610K and 660W arrays, as described previously.[28] We examined single nucleotide polymorphisms (SNPs) for PON1–Q192, which was directly genotyped, and four other SNPs that were imputed in the genotype data set, including rs705379 (PON1–108), rs705381 (PON1–161), rs854560 (PON1–L55M), and rs854572 (PON1–909). MaCH 1.0[29] was used to impute the 1000 Genomes Iv3 reference panel,[30] and all four imputed SNPs had excellent imputation quality (R2>0.95) and high minor allele frequencies (>26%). For individuals with no genotyping data available, all SNPs were imputed as described below.

Statistical methods

All analyses were performed using R (version 3.4.3; R Development Core Team).[31] To address missing data, we imputed the data 10 times using multiple imputation by chained equation (MICE) in R (package mice).[32] For DAPs, a small number of concentrations were missing due to insufficient sample or machine error (≤5 measurements for any visit for DMPs; ≤23 for DEPs; ≤5 for creatinine). Imputations were performed prior to calculating nanomolar sums, creatinine correction, and calculation of subject-specific averages. Missing covariates listed in Table 1 (<20% for all) were imputed, and the

following covariates were additionally included as predictors for imputation: maternal education level; caloric intake; caloric intake from vegetables and caloric intake from fruits; paternal education level; maternal ethnicity; and body mass index (BMI). We also imputed missing ultrasound and delivery SDS of fetal or newborn size. Due to the correlation between size measurements, only birth-weight SDS was included as a predictor in the MICE procedure. Finally, for individuals missing all PON1 genotyping data, we imputed SNPs in the same MICE procedure as has been done in our previous analyses; however, PON1 was not used as a predictor in the imputation step due to a high proportion of missing measures. Thus, unless stated otherwise, all models presented contain the full sample (n=784) and complete observations at all time points.

We calculated distributions of demographic characteristics and DAP averages and examined Pearson correlations between DAPs at individual time points and for averages. We calculated raw (i.e., unstandardized) ultrasound and delivery measures of size in the unimputed data set for interpretation purposes. To address our primary research question, we created linear mixed effects models using the nlme package,[33] modeling average DMP, DEP, or DAP exposure over pregnancy in relation to repeated SDS of head circumference, length, or weight (ultrasound measures from middle and late pregnancy in combination with birth measurements at delivery). In this and other pregnant populations, within-individual variation in urinary DAP concentrations is greater than variation between individuals.[34,35] This reflects daily variation in exposure through, for example, variable dietary patterns, as well as rapid metabolic clearance of these compounds.[13] Consequently, if exposures are relatively consistent over longer periods of time, average DAP concentrations based on multiple urine samples should provide a more accurate measure of usual exposure at any point during pregnancy than DAP concentrations measured in an individual sample. Therefore, in our primary analyses, we used pregnancy average DAP concentrations to estimate usual exposure across pregnancy despite the fact that this included urinary biomarkers from late pregnancy that were collected after the mid-pregnancy ultrasound. DAP averages were log10-transformed for analysis to improve model fit. All models included a random intercept for each subject as well as a random slope for gestational age at growth measurement. We additionally included an interaction term between DAP average and gestational age at growth measurement to allow the exposure–outcome association to differ by timing of outcome measurement. Because results differed based on the timing of outcome measurement and because presentation of main effect and interaction terms can be difficult to interpret, we presented results from models where the intercept was varied so that the results would represent associations where the outcome was measured at 20, 30, and 40 weeks of gestation.

For adjusted models, we included an a priori set of covariates based on previously observed associations with the exposure and outcome or covariates known to improve

the precision of the outcome estimate. These included fetal sex (categorical), maternal age (continuous), prepregnancy weight (continuous), height (continuous), maternal education level (categorical), maternal ethnicity (categorical), parity (categorical), smoking in pregnancy (categorical), alcohol use in pregnancy (categorical), folic acid use, and gestational age at growth measurement (continuous). Folic acid was included in this study population because it has been associated with fetal growth and because it is a strong indicator of socioeconomic status (SES), a predictor of urinary DAP concentrations.[13,36,37] Maternal height and weight were included instead of the aggregate BMI because each is an independent predictor of fetal growth.[38]

Our second aim was to examine windows of vulnerability to exposure. To do so, we created cross-sectional models of total urinary DAP concentrations measured at each study visit in relation to outcome measurements at middle and late pregnancy and at delivery (i.e., one exposure time point and one outcome time point per model). For these analyses, we examined only outcomes at the same or subsequent visits (i.e., we did not model late pregnancy exposure biomarkers in association with middle pregnancy fetal growth measurements). All models retained the same covariates as those used in the repeated measures analyses. Results for all cross-sectional models are reported for associations with outcomes at early, middle, or late pregnancy and are referred to as such. To assess effect modification of the relationship between DAPs and fetal growth by fetal sex, we examined associations in models stratified by this variable, and we additionally included a two-way interaction term between sex and exposure in repeated measures models to test the significance of any observed differences. A similar approach was used to estimate effect modification by PON1 genotype. Interaction terms with $p<0.05$ were considered statistically significant.

To test the robustness of our results, we first examined the influence of imputing outcome measurements. To do so, we created a new set of 10 imputed data sets in which the low proportion missing for exposures and covariates were imputed, but the outcome was not. We then recreated linear mixed effects models for comparison. Second, we examined results with DMPs and DEPs included in the same model in order to distinguish the effects of the two classes. Third, because these metabolites demonstrate only weak-to-moderate reliability over pregnancy (e.g., intraclass correlation coefficient for DAP metabolites=0.30),[35] we examined the effect of adjusting for measurement error by applying regression calibration.[39] We applied the calibration to repeated measures models of average DAP concentrations in associations with each fetal growth outcome. Fourth, to test the robustness of our results to adjustment for additional SES factors, we examined models additionally adjusted for marital status and income level. Fifth, because season has been associated both with urinary DAP[13] and with birth weight in some studies,[40] we examined the effect of additionally adjusting for this potential confounder. Finally,

we examined associations after removing babies who were born preterm (i.e., prior to 37 weeks of completed gestation) in order to determine whether or not our results could be attributed to gestational age at delivery rather than size.

Results

Of the 784 women included in the present analysis, the median maternal age was 31 y, most of the women were Dutch (58%), and the prepregnancy median weight was 64kg (Table 1). For 62% of women this was their first pregnancy, and smoking and alcohol use was low to moderate in the study population. A small percentage of women never took folic acid supplements either prior to or at any point during pregnancy.

The median gestational ages for middle and late pregnancy ultrasounds were 20.4 weeks [95% confidence interval (CI): 20.3, 20.5] and 30.4 weeks (95% CI: 30.3, 30.5), respectively, and the median gestational age at delivery was 40 weeks (95% CI: 40.0, 40.2) (Table 2).

Almost all participants included in the present analysis had ultrasound measurements available at these two time points prior to imputation. All participants had data available on birth weight at delivery, but a smaller proportion had head circumference (61%) or birth length (72%) assessed. Thus, a larger proportion of head circumference and birth length measurements were imputed. Distributions of urinary DMPs, DEPs, and DAPs by study visit and on average are presented in Table 3. As previously reported, concentrations by visit showed weak-to-moderate reliability (intraclass correlation coefficients 0.14–0.38).[35] DMPs and DAPs were highly correlated both for averages and at individual study visits (Pearson r=0.97–0.98), but DEPs were less correlated with DAPs (Pearson r=0.53–0.63) and DMPs (Pearson r=0.43–0.47) (see Table S1).

Primary analysis: repeated measures models

Effect estimates from fully adjusted repeated measures models, accounting for interaction between exposure and gestational age at growth measurement, demonstrated that associations between pregnancy averages of exposure and outcomes differed based on the timing of outcome measurement (i.e., interactions between exposure and time were statistically significant; see Table S2). For the presentation of results, we calculated effect estimates for outcomes at 20, 30, and 40 weeks (Table 4).

At 20 weeks, a 10-fold increase in pregnancy-averaged total DAPs was associated with a 0.53-SDS shorter length (95% CI: −0.83, −0.23) and a 0.32-SDS lower weight (95% CI: −0.59, −0.04). For length, this difference corresponds to −2mm, or −5%, relative to the

Table 1. Demographic and lifestyle characteristics of 784 mothers with singleton live births from the Generation R study population.

		Median (25th, 75th) or N (%)
Maternal age in years		31 (28, 34)
	<20	14 (1.79)
	20- <25	79 (10.1)
	25- <30	208 (26.5)
	30- <35	360 (45.9)
	>=35	123 (15.7)
Maternal ethnicity		
	Dutch	451 (57.5)
	Other western	70 (8.93)
	Non-western	263 (33.6)
Maternal education		
	Low	113 (14.9)
	Intermediate	229 (30.2)
	High	417 (54.9)
	missing	25 (3.12)
Household income in Euros		
	<1200 per month	86 (11.0)
	1200-2000 per month	113 (14.4)
	>2000 per month	483 (61.6)
	missing	102 (13.0)
Marital status		
	Partner	677 (89.7)
	No partner	78 (10.3)
	missing	29 (3.70)
	Weight pre-pregnancy in kg	64.0 (58.0, 72.0)
	missing	96 (12.2)
Height at visit 1 in cm		168 (163, 173)
	missing	1 (0.13)
Parity		
	0	486 (62.0)
	1	208 (26.5)
	>=2	86 (11.0)
	missing	4 (0.51)
Smoking		
	No smoking during pregnancy	555 (70.8)
	Until pregnancy recognized	64 (8.16)
	Continued during pregnancy	102 (13.0)
	missing	63 (8.04)
Alcohol consumption		
	No consumption during pregnancy	273 (34.8)
	Until pregnancy recognized	130 (16.6)
	Continued occasionally	293 (37.4)
	Continued frequently	48 (6.12)
	missing	40 (5.10)
Folic acid intake		
	None	98 (12.5)
	Started in first 10 weeks of pregnancy	212 (27.0)
	Start pre-conception	319 (40.7)
	missing	155 (19.8)
Fetal sex		
	Male	398 (50.8)
	Female	386 (49.2)
Preterm (<37 weeks of gestation)		
	No	762 (97.1)
	Yes	22 (2.90)
Low birth weight (<2500 grams)		
	No	761 (97.0)
	Yes	23 (3.0)

Table 2. Distribution of fetal and neonatal anthropometric parameters (unimputed).

	N (%)	Mean (SD)
Middle pregnancy (ultrasound)		
Gestational age (weeks)	784 (100)	20.4 (0.92)
Head circumference (mm)	774 (98.7)	178 (12.3)
Femur length (mm)	779 (99.4)	33.1 (2.98)
Estimated fetal weight (grams)	777 (99.1)	369 (73.8)
Late pregnancy (ultrasound)		
Gestational age (weeks)	784 (100)	30.4 (0.83)
Head circumference (mm)	777 (99.1)	286 (11.4)
Femur length (mm)	784 (100)	57.6 (2.80)
Estimated fetal weight (grams)	782 (99.7)	1626 (238)
Delivery (physical exam)		
Gestational age (weeks)	784 (100)	40.1 (1.48)
Birth head circumference (cm)	478 (61)	33.7 (1.72)
Birth length (cm)	562 (71.7)	50.4 (2.24)
Birth weight (g)	784 (100)	3452 (505)

Table 3. Distribution of organophosphate pesticide metabolite concentrations by study visit and on average (nmol/g creatinine).

		Percentiles			
	Geometric Mean	25th	50th	75th	95th
Dimethyl phosphates (DMPs)[a]					
Early pregnancy	249.8	148.6	244.1	413.6	860.0
Middle pregnancy	263.6	168.8	268.6	415.4	854.3
Late pregnancy	247.4	157.1	247.8	398.9	863.1
Average	253.5	183.1	259.1	355.3	582.9
Diethyl phosphates (DEPs)[b]					
Early pregnancy	42.9	25.1	43.1	79.3	175.6
Middle pregnancy	40.5	23.2	41.6	74.3	179.8
Late pregnancy	40.1	21.6	41.5	77.3	176.3
Average	40.0	28.2	42.6	64.6	116.6
Dialkyl phosphates (DAPs)[c]					
Early pregnancy	308.4	188.1	306.9	499.3	989.0
Middle pregnancy	317.9	206.7	316.5	485.9	1001.9
Late pregnancy	301.5	194.0	307.9	489.0	984.8
Average	309.2	226.4	311.0	438.8	687.3

[a]DMPs represent a molar sum of dimethylphosphate (DMP), dimethylthiophosphate (DMTP), and dimethyldithiophosphate (DMDTP). [b]DEPs represent a molar sum of diethylphosphate (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP). [c]DAPs represent a molar sum of all of the above. Percent below the limit of detection by individual metabolite in early, middle, and late pregnancy: DMDTP: 19.9, 18.1, 18.0; DMP: 0.1, 0, 0; DMTP: 3.5, 3.6, 2.4; DEDTP: 81.1, 84.5, 85.0; DEP: 2.7, 5.4, 4.1; DETP: 12.1, 11.8, 11.7. Number of metabolites missing due to machine error in early, middle, and late pregnancy: DMDTP: 0, 0, 3; DMP: 0, 0, 0; DMTP: 0, 0, 1; DEDTP: 0, 1, 1; DEP: 1, 0, 0; DETP: 13, 22, 16. Number of metabolites missing due to insufficient urine volume for analyses: early pregnancy=5; middle pregnancy=1; late pregnancy=1 (6 participants total).

mean for length at 20 weeks of gestation. For weight, this corresponds to –24g, or –6%, relative to the mean for estimated fetal weight at 20 weeks of gestation. DMPs and DEPs individually were also inversely associated with length and weight at this time point, but none of the DAPs were significantly associated with differences in head circumference. Interaction terms between exposure and gestational age indicated that associations became weaker as pregnancy progressed, so that at 30 or 40 weeks no significant associations between DAPs and fetal measurements were observed (Table 4).

To illustrate these effects, we plotted estimated coefficients and confidence intervals by time for associations between DAPs and head circumference (Figure 1A), length (Figure 1B), and weight (Figure 1C). This shows that at delivery associations were null for weight and positive but nonsignificant for head circumference and length. These results are also consistent with those from cross-sectional models of pregnancy averages in association with growth measurements from each study visit (middle pregnancy, late pregnancy, and delivery). Cross-sectional associations with pregnancy averages are displayed in Figure 2D, with effect estimates in Table S3.

Table 4. Adjusted[a] difference in fetal head circumference, length, or weight standard deviation (SDS) score at selected weeks gestation in association with pregnancy average urinary organophosphate pesticide metabolite concentrations.

		Total DMPs	Total DEPs	Total DAPs
		Difference in SDS score (95% CI)	Difference in SDS score (95% CI)	Difference in SDS score (95% CI)
Head circumference				
	20 weeks	-0.11 (-0.42, 0.19)	0.00 (-0.13, 0.14)	-0.09 (-0.40, 0.23)
	30 weeks	0.12 (-0.14, 0.38)	0.07 (-0.04, 0.18)	0.13 (-0.14, 0.39)
	40 weeks	0.35 (-0.07, 0.78)	0.13 (-0.06, 0.33)	0.34 (-0.11, 0.79)
Length				
	20 weeks	-0.46 (-0.75, -0.17)	-0.17 (-0.30, -0.04)	-0.53 (-0.83, -0.23)
	30 weeks	-0.09 (-0.32, 0.14)	-0.06 (-0.17, 0.04)	-0.13 (-0.37, 0.10)
	40 weeks	0.29 (-0.05, 0.63)	0.05 (-0.11, 0.21)	0.27 (-0.08, 0.61)
Weight				
	20 weeks	-0.27 (-0.54, 0.00)	-0.16 (-0.28, -0.04)	-0.32 (-0.59, -0.04)
	30 weeks	-0.10 (-0.32, 0.12)	-0.08 (-0.18. 0.02)	-0.13 (-0.36, 0.09)
	40 weeks	0.06 (-0.22, 0.35)	0.00 (-0.13, 0.13)	0.05 (-0.25, 0.34)

[a]Results from linear mixed effects models adjusted for fetal sex, maternal age (continuous), pre-pregnancy weight (continuous), height (continuous), education level (categorical), maternal ethnicity (categorical), parity (categorical), smoking (categorical), alcohol use (categorical), folic acid use (categorical), and gestational age at ultrasound or delivery (continuous). Models contain an interaction term between exposure concentration and gestational age at ultrasound/delivery, a random intercept for each participant, and a random slope for gestational age at ultrasound/delivery. Abbreviations: DMPs, dimethyl phosphates; DEPs, diethyl phosphates; DAPs, dialkyl phosphates; CI, confidence interval.

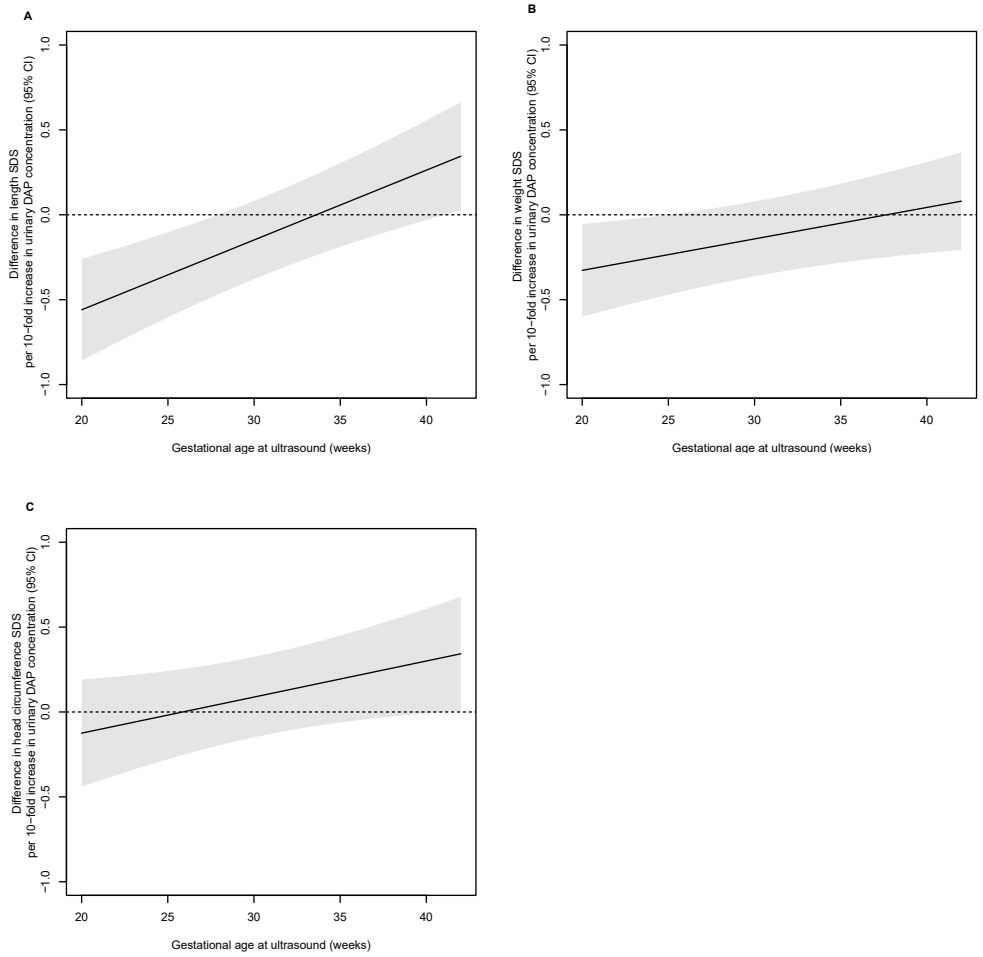

Figure 1. Adjusted repeated measures associations between pregnancy average total dialkyl phosphate (DAP) concentrations and standard deviation scores (SDS) of (A) head circumference, (B) length, and (C) weight by gestational age at growth measurement in the Generation R Study population (n=784). Models adjusted for fetal sex, maternal age (continuous), prepregnancy weight (continuous), height (continuous), education level (categorical), maternal ethnicity (categorical), parity (categorical), smoking (categorical), alcohol use (categorical), folic acid use (categorical), and gestational age at ultrasound or delivery (continuous). Model contains an interaction term between exposure concentration and gestational age at ultrasound/delivery, a random intercept for each participant, and a random slope for gestational age at ultrasound/delivery. Main effect and interaction terms (95% CIs) for each plot are as follows: (A) −0.51 (−1.32, 0.29); 0.02 (−0.01, 0.05); (B) −1.33 (−2.00, −0.66); 0.04 (0.02, 0.06); (C) −0.68 (−1.24, −0.12); 0.02 (0.00, 0.04). This figure includes imputed data. Note: CI, confidence interval; DAPs, dialkyl phosphates; SDS, standard deviation scores.

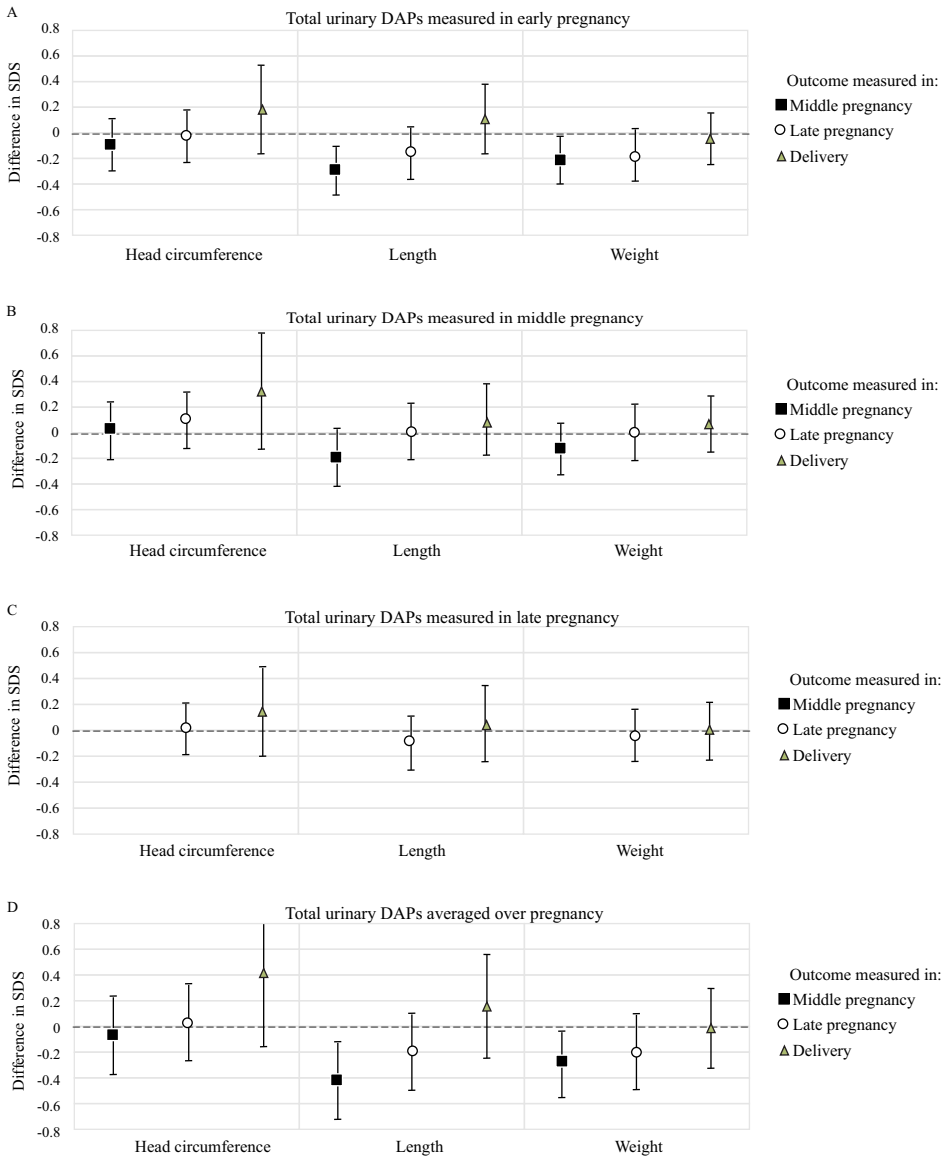

Figure 2. Adjusted cross-sectional associations between visit-specific total dialkyl phosphate (DAP) concentrations measured in total urinary DAPs in A) early pregnancy, B) middle pregnancy, C) late pregnancy, and D) averaged over pregnancy and standard deviation scores (SDS) of fetal growth parameters (head circumference, length, and weight) measured during pregnancy by ultrasound and by clinical examination at delivery in the Generation R Study population (n=784). Model adjusted for fetal sex, maternal age (continuous), prepregnancy weight (continuous), height (continuous), education level (categorical), maternal ethnicity (categorical), parity (categorical), smoking (categorical), alcohol use (categorical), folic acid use (categorical), and gestational age at ultrasound or delivery (continuous). This figure includes imputed data. Note: CI, confidence interval; DAPs, dialkyl phosphates; SDS, standard deviation scores.

Secondary analysis: windows of vulnerability to exposure

Cross-sectional models of visit-specific urinary DAP concentrations in association with each outcome demonstrated some differences by timing of exposure (Figure 2A–C; see also Tables S4–S6). In general, total urinary DAPs measured in early pregnancy showed the strongest associations with length and weight (Figure 2A; Table S4). A 10-fold increase in concentrations measured in samples collected in early pregnancy was associated with lower fetal length (β=–0.30, 95% CI: –0.50, –0.10) and weight (β=–0.22, 95% CI: –0.4, –0.04) at mid-pregnancy, and also with lower fetal length (β=–0.16, 95% CI: –0.36, 0.04) and weight (β=–0.19, 95% CI: –0.39, 0.01) in late pregnancy, although the latter association was not statistically significant. Levels measured in mid-pregnancy samples were associated with lower fetal length (β=–0.2, 95% CI: –0.42, 0.02) and weight (β=–0.14, 95% CI: –0.34, 0.06) in mid-pregnancy but not in late pregnancy or at delivery (Figure 2B; Table S5). Finally, levels measured in late pregnancy samples were not associated with differences in length or weight but were positively associated with head circumference measured at delivery (Figure 2C; Table S6). Patterns for DMPs and DEPs were similar to the overall DAPs (see Figures S2–S3 and Tables S4–S6).

Effect modification by sex and genotype

Interaction terms between total DAPs and sex demonstrated associations with length and weight that were stronger (i.e., more negative) for males compared with females (Table 5), although associations were still observed for females in models of length. Adjusted associations by gestational age and sex are displayed in Figures 3A–C for length, weight, and head circumference respectively. These figures show how the exposure–response associations differ in males and females based on timing of outcome measurement. Interaction terms between total DAPs and PON1 genotype were not statistically significant (p=0.18–0.93) and there were no clear differences in exposure–outcome associations according to SNP genotypes (see Tables S7A–E).

Sensitivity analyses

For repeated measures models, associations without adjustment for covariates were slightly greater in magnitude (i.e., more negative) but otherwise similar to those observed in our primary analysis (see Table S8). Associations in models without imputed outcome were also similar, although effect estimates for head circumference were closer to the null (see Table S9). Summed DEPs and DMPs were moderately correlated (Pearson r=0.45). When exposures were both included in a mutually adjusted model, associations were similar to the primary single-pollutant findings (see Table S10). In both, effect estimates were greater in magnitude (i.e., more negative) for DMPs compared with DEPs for all growth parameters. We additionally examined the effect of adjusting for the measurement error inherent in our exposure biomarkers by creating our repeated measures models of average urinary DAPs with application of regression calibration. As expected, we observed that

Table 5. Adjusted difference in fetal head circumference, length, or weight standard deviation score (SDS) at selected weeks gestation in association with pregnancy average urinary organophosphate pesticide metabolite concentrations in a subset of the Generation R Study population from models stratified by infant sex (n =398 males, 386 females).

	Total DMPs			Total DEPs			Total DAPs		
	Male	Female	Pint[a]	Male	Female	Pint[a]	Male	Female	Pint[a]
Head circumference									
20 weeks	-0.13 (-0.57, 0.31)	-0.20 (-0.67, 0.28)	0.25	0.06 (-0.12, 0.25)	-0.14 (-0.37, 0.09)	0.80	-0.11 (-0.56, 0.35)	-0.18 (-0.67, 0.30)	0.36
30 weeks	-0.01 (-0.36, 0.34)	-0.23 (-0.13, 0.59)		0.07 (-0.07, 0.22)	0.04 (-0.13, 0.21)		0.02 (-0.35, 0.39)	0.20 (-0.16, 0.57)	
40 weeks	0.10 (-0.43, 0.64)	-0.66 (0.06, 1.25)		0.08 (-0.16, 0.32)	0.22 (-0.04, 0.47)		0.15 (-0.42, 0.71)	0.59 (0.00, 1.18)	
Length									
20 weeks	-0.79 (-1.22, -0.37)	-0.18 (-0.63, 0.27)	0.02	-0.16 (-0.34, 0.02)	-0.23 (-0.45, 0.00)	0.49	-0.84 (-1.27, -0.40)	-0.27 (-0.74, 0.19)	0.03
30 weeks	-0.38 (-0.69, -0.07)	0.14 (-0.20, 0.48)		-0.04 (-0.17, 0.10)	-0.14 (-0.31, 0.02)		-0.40 (-0.72, -0.07)	0.07 (-0.28, 0.42)	
40 weeks	0.03 (-0.43, 0.49)	0.46 (-0.01, 0.92)		0.08 (-0.12, 0.29)	-0.06 (-0.29, 0.17)		0.04 (-0.43, 0.51)	0.42 (-0.06, 0.90)	
Weight									
20 weeks	-0.61 (-1.00, -0.22)	0.07 (-0.32, 0.46)	<0.01	-0.16 (-0.32, 0.00)	-0.18 (-0.38, 0.01)	0.67	-0.65 (-1.06, -0.25)	0.02 (-0.38, 0.43)	0.01
30 weeks	-0.37 (-0.67, -0.07)	0.15 (-0.17, 0.46)		-0.06 (-0.18, 0.06)	-0.14 (-0.30, 0.01)		-0.40 (-0.71, -0.09)	0.11 (-0.21, 0.44)	
40 weeks	-0.13 (-0.51, 0.24)	0.22 (-0.19, 0.63)		0.04 (-0.11, 0.20)	-0.11 (-0.32, 0.10)		-0.15 (-0.54, 0.24)	0.20 (-0.22, 0.62)	

Note. Models adjusted for maternal age (continuous), pre-pregnancy weight (continuous), height (continuous), education level (categorical), maternal ethnicity (categorical), parity (categorical), smoking (categorical), alcohol use (categorical), folic acid use (categorical), and gestational age at ultrasound or delivery (continuous). Models contain an interaction term between exposure concentration and gestational age at ultrasound/delivery, a random intercept for each participant, and a random slope for gestational age at ultrasound/delivery. Abbreviations: DMPs, dimethyl phosphates; DEPs, diethyl phosphates; DAPs, dialkyl phosphates; CI, confidence interval. pint, p for interaction. Values in this table include imputed data.

a. p-values for the interaction term between exposure concentration and sex.

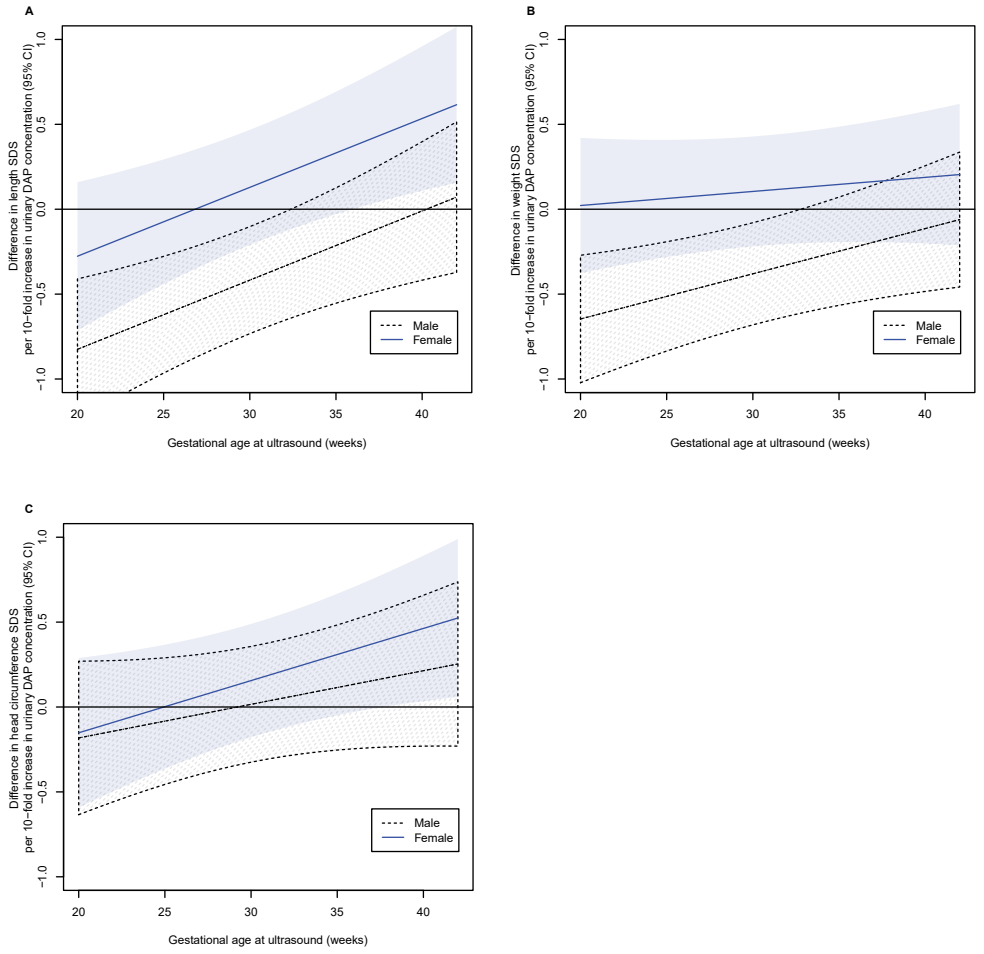

Figure 3. Adjusted and sex-stratified repeated measures associations between pregnancy average total dialkyl phosphate (DAP) concentrations and standard deviation scores of (A) head circumferences, (B) length, and (C) weight by gestational age at growth measurement in the Generation R Study population (n=784, 398 males 386 females). Model adjusted for maternal age (continuous), prepregnancy weight (continuous), height (continuous), education level (categorical), maternal ethnicity (categorical), parity (categorical), smoking (categorical), alcohol use (categorical), folic acid use (categorical), and gestational age at ultrasound or delivery (continuous). Models contained an interaction term between exposure concentration and gestational age at ultrasound/delivery, a random intercept for each participant, and a random slope for gestational age at ultrasound/delivery. Main effect and interaction terms (95% CIs) for each plot are as follows: (A) male: −0.35 (−1.35 , 0.66); 0.01 (−0.02 , 0.05); female: −0.92 (−2.06 , 0.22); 0.04 (0.00, 0.08); (B) male: −1.67 (−2.62 , −0.72); 0.04 (0.01, 0.08); female: −0.93 (−1.90 , 0.04); 0.03 (0.00, 0.07); (C) male: −1.13 (−1.93 , −0.33); 0.03 (0.00, 0.05); female: −0.15 (−0.93 , 0.64); 0.01 (−0.02 , 0.03). This figure includes imputed data. Note: CI, confidence interval; DAPs, dialkyl phosphates; SDS, standard deviation scores.

the effect estimates were less attenuated (i.e., farther from the null) compared with the results from the primary model but that variances were increased (see Table S11). Finally, we observed minimal differences when we added marital status and family income as additional measures of SES to the model, when we included season of sample collection, or when our population was restricted to babies born at term (see Table S12–S14, respectively, for DAPs only).

Discussion

In a study of pregnant women in the Netherlands, we observed that elevated urinary biomarkers of OP exposure were associated with reduced fetal size as indicated by measurements of length and weight in mid-pregnancy, but not at delivery. Furthermore, elevated biomarkers of exposure from early as well as mid-pregnancy demonstrated associations with growth measurements that were greatest in magnitude and the most precise. The latter suggests that early pregnancy exposure may be an important vulnerable window for the relationship between OP exposure and fetal growth.

The results from our analysis may shed light on previous epidemiologic studies with ambiguous findings on this relationship. All previous studies have examined exposure in association with measurements at delivery, and few have found evidence of effects. This is consistent with what we would expect based on our study given that we observed no associations between exposure biomarkers and outcomes measured at delivery. A recent pooled study combined data from four U.S. studies for a powerful assessment of this research question (total sample size~1,100); however, no association was observed overall between prenatal urinary exposure biomarkers and birth weight, length, or head circumference.[21] Other individual studies using urinary biomarkers have noted some associations between these exposures and outcomes, with differences by timing of exposure assessment, PON1 genotype or expression, and, in some instances, fetal sex; however, no clear patterns emerge upon review of the data.[41-49]

Some notable differences exist between this previous work and our present study. First, all but two of the previous studies had significantly smaller sample sizes (n~50–450). However, those with similar sample sizes had null findings. The largest study to date was the pooled analysis, although the authors of that study noted the difficulties in combining these data across populations with differing demographics and exposure levels (n~1,000).[21] The second largest study from the Odense Child Cohort in Denmark (n=858) measured urinary DAP concentrations at ~28 weeks of gestation and was unable to detect associations with birth weight, length, or abdominal or head circumference at delivery.[47] Second, all but three studies assessed urine concentrations in a single spot urine sample collected

during gestation or at delivery. Those with repeated measures had largely null findings, although they were also more limited in sample size. Woods et al. (n=272) averaged measures from 16 and 26 weeks of gestation and did not detect associations with birth weight.[49] Naksen et al. (n=52) and Huang et al. (n=105) modeled urinary concentrations from two visits during pregnancy and at delivery separately, with primarily null results.[43,48] OPs are metabolized quickly in the human body, and urinary DAP concentrations show only moderate stability over pregnancy;[13,48] thus, the availability of repeated measures for estimating more stable subject-specific averages is an advantage in our study. Even though these averages included a urinary measurement (late pregnancy) that was taken after the time of some outcome measurements (ultrasound measures from mid-pregnancy), the average is the best choice for exposure assessment because individual measurements are highly variable over time due to variations in exposure sources (e.g., diet) in combination with rapid metabolic excretion.[34,35] Consequently, if we assume that exposures are generally consistent over pregnancy, the average measure will be the best estimate.

In addition, availability of repeated measurements enabled us to investigate windows of vulnerability to exposure during gestation. We observed that urinary DAP concentrations from early pregnancy were associated with reduced length and estimated fetal weight in mid- and late pregnancy. DAP levels from mid- and late pregnancy, however, were not associated with growth measurements. This could suggest that early pregnancy is a particularly sensitive window to exposure. Early to mid-pregnancy is a time of rapid placental development that could be mediating these effects.

A third major difference between our study and those previously published is in exposure biomarker levels, which differ greatly across these populations. The average urinary DAP concentration in this study population was 312 nmol/g creatinine, whereas levels in most studies range from 10–100 nmol/g creatinine. Levels may be higher in the Generation R cohort participants due to a higher consumption of fruits in this study population or to the application of larger quantities of pesticides in farms in the Netherlands.[13] The only study to note higher levels was the abovementioned study by Huang et al. (2017), which reported geometric mean concentrations of summed DMP and DEP metabolites as high as 569 and 282 nmol/g creatinine, respectively, in samples analyzed at 11 and 26 weeks of gestation and at delivery. It is not apparent that studies with higher exposure levels are more likely to demonstrate an association with differences in birth outcomes at delivery; however, this might be difficult to detect if all members of the given population are more highly exposed.

Associations observed between urinary DAPs and fetal length and weight in mid-pregnancy were more pronounced in males compared with females. Similar sex differences have been observed in associations between this exposure and neurobehavioral deficits, with males

demonstrating stronger associations.[50,51] Placental differences by sex, including epigenetic patterns,[52] could influence the amount of the toxic compound that is transferred to the fetus and partially explain these differences. Alternatively, the differences observed may be due to the fact that the male fetus is more vulnerable to adverse pregnancy outcomes, particularly to perturbations during their rapid growth in early pregnancy.[53,54]

The findings from the present study may be difficult to interpret clinically because no associations were detected with size at delivery. However, differences in growth in early pregnancy may be crucial for health outcomes later in life. First-trimester growth restriction is associated with faster weight gain and adverse cardiovascular profiles in school-age children.[55,56] This may be a particularly sensitive time in development, and the consequences of the associations we observed should be investigated in future work.

Our ability to detect differences in growth in association with exposure early in pregnancy could be due to methodological issues as well. Estimated fetal weight as calculated by a combination of ultrasound measures is subject to much more measurement error compared with birth weight.[57] However, we would predict that this would lead to improved ability to detect associations between exposure and weight at delivery rather than early in pregnancy. Alternatively, because fetal weight gain occurs primarily in the third trimester, the influence of any error in the estimate of gestational age might be more pronounced toward the end of pregnancy. This could partially explain this difference in our findings based on timing of outcome measurement.

The primary limitation of this study is the nonspecificity of DAPs. Because OPs are metabolized rapidly, these biomarkers remain the best and most commonly used indices of total individual-level exposure.[58] However, DAPs reflect human exposure to both the toxic compounds as well as their nontoxic metabolites, which are formed outside the body and can enter the human body through the same exposure routes as the parent compounds. Urinary concentrations of DAPs, therefore, may overestimate exposure to the toxic compounds of interest. This may mean, however, that the associations observed with DAPs in this and other studies may be lower than those that would have been observed with a better estimator of exposure.[59]

In general, our estimates of exposure are superior to those from other human studies because we measured spot urine concentrations of DAPs at three time points in pregnancy, creating subject-specific averages that may be a more stable reflection of exposure over time.[35] Despite this improvement, the measurement error may have biased our effect estimates toward the null. Indeed, adjusting for measurement error with regression calibration resulted in effect estimates that were farther from the null but more imprecise, illustrating the known trade-off between bias and variance.[60]

Our study was also limited by smaller sample sizes with available information on head circumference and body length on neonates. However, we handled missingness by imputing using the MICE procedure, which we previously showed was a suitable approach for fetal growth data.[61] In addition, associations in an unimputed data set were very similar to those shown in our primary results, with the exception of the associations between DAPs and head circumference, which were closer to the null. Last, combining measures of fetal size with measures at delivery in repeated measures models may be problematic because they are measured differently and hence reflect different outcomes. This could be another explanation for the differences we observed between associations with ultrasound measurements in mid-pregnancy vs. anthropometric measurements at delivery. However, we also observed associations closer to the null at late pregnancy with measurements also taken by ultrasound, so we believe this is unlikely to be the case.

The major strengths of our study were the large sample size, the availability of three urinary measurements of DAP metabolites to assess exposure, and the use of repeated ultrasound scans that captured fetal size at multiple time points in pregnancy and in different parameters (e.g., length in addition to weight). This allowed us to investigate associations with OP exposure during gestation that have not been previously examined and enabled detection of decreased fetal growth in early pregnancy in association with exposure.

In summary, urinary biomarkers of OPs were inversely associated with length and weight in mid-pregnancy, with stronger associations observed for exposure biomarkers measured in urine samples collected during early and mid-pregnancy as well as with stronger associations observed in males compared with females. Future research should be directed toward improving the understanding of the consequence of these differences observed on health outcomes later in life.

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Supplemental Material

Table S1. Pearson correlations between log10 transformed DAP metabolite concentrations from early, middle, and late pregnancy as well as on average (N=784).

		DAPs				DEPs				DMPs		
		Early	Mid	Late	Mean	Early	Mid	Late	Mean	Early	Mid	Late
DAPs												
	Early	-										
	Mid	0.26	-									
	Late	0.18	0.34	-								
	Mean	0.68	0.74	0.71	-							
DEPs												
	Early	0.63	0.20	0.14	0.46	-						
	Mid	0.24	0.61	0.23	0.50	0.24	-					
	Late	0.16	0.30	0.61	0.50	0.15	0.27	-				
	Mean	0.40	0.41	0.33	0.53	0.61	0.64	0.59	-			
DMPs												
	Early	0.97	0.23	0.16	0.65	0.47	0.20	0.14	0.31	-		
	Mid	0.24	0.98	0.34	0.72	0.17	0.47	0.28	0.34	0.22	-	
	Late	0.16	0.33	0.97	0.68	0.12	0.21	0.46	0.26	0.15	0.32	-
	Mean	0.67	d0.72	0.70	0.98	0.37	0.41	0.42	0.43	0.67	0.73	0.70

Table S2. Main effects and interaction terms from adjusteda repeated measures models of associations between pregnancy average urinary organophosphate pesticide metabolite concentrations and standard deviation scores of fetal growth parameters measured during pregnancy by ultrasound and by clinical examination at delivery.

		Main effect	Interaction with gestational age
		ß (95% CI)	ß (95% CI)
Head circumference			
	Total DMPs	-0.58 (-1.34, 0.19)	0.02 (0.00, 0.05)
	Total DEPs	-0.13 (-0.48, 0.22)	0.01 (-0.01, 0.02)
	Total DAPs	-0.51 (-1.32, 0.29)	0.02 (-0.01, 0.05)
Length			
	Total DMPs	-1.20 (-1.86, -0.54)	0.04 (0.02, 0.06)
	Total DEPs	-0.39 (-0.70, -0.08)	0.01 (0.00, 0.02)
	Total DAPs	-1.33 (-2.00, -0.66)	0.04 (0.02, 0.06)
Weight			
	Total DMPs	-0.60 (-1.15, -0.05)	0.02 (0.00, 0.03)
	Total DEPs	-0.32 (-0.57, -0.08)	0.01 (0.00, 0.02)
	Total DAPs	-0.68 (-1.24, -0.12)	0.02 (0.00, 0.04)

a Models adjusted for fetal sex, maternal age (continuous), pre-pregnancy weight (continuous), height (continuous), education level (categorical), ethnicity (categorical), parity (categorical), smoking (categorical), alcohol use (categorical), folic acid use (categorical), and gestational age at ultrasound or delivery (continuous). Models contain an interaction term between exposure concentration and gestational age at ultrasound/delivery, a random intercept for each participant, and a random slope for gestational age at ultrasound/delivery. Abbreviations: DMPs, dimethyl phosphates; DEPs, diethyl phosphates; DAPs, dialkyl phosphates; CI, confidence interval.

Table S3. Adjusted[a] cross-sectional associations between pregnancy average urinary organophosphate pesticide metabolite concentrations and standard deviation scores of fetal growth parameters measured during pregnancy by ultrasound and at delivery.

	Total DMPs	Total DEPs	Total DAPs
	Difference in SDS score (95% CI)	Difference in SDS score (95% CI)	Difference in SDS score (95% CI)
Head circumference			
Middle pregnancy	-0.06 (-0.36, 0.23)	0.03 (-0.10, 0.16)	-0.06 (-0.36, 0.25)
Late pregnancy	0.01 (-0.28, 0.30)	0.02 (-0.11, 0.15)	0.04 (-0.26, 0.34)
Delivery	0.44 (-0.09, 0.98)	0.15 (-0.06, 0.37)	0.43 (-0.14, 1.00)
Length			
Middle pregnancy	-0.35 (-0.64, -0.06)	-0.11 (-0.23, 0.02)	-0.40 (-0.70, -0.10)
Late pregnancy	-0.13 (-0.42, 0.15)	-0.10 (-0.23, 0.02)	-0.19 (-0.48, 0.11)
Delivery	0.20 (-0.20, 0.60)	0.01 (-0.18, 0.20)	0.17 (-0.24, 0.57)
Weight			
Middle pregnancy	-0.22 (-0.49, 0.05)	-0.13 (-0.25, -0.01)	-0.26 (-0.54, 0.01)
Late pregnancy	-0.14 (-0.42, 0.14)	-0.12 (-0.24, 0.01)	-0.18 (-0.47, 0.11)
Delivery	0.02 (-0.28, 0.31)	0.00 (-0.14, 0.13)	0.00 (-0.31, 0.31)

a Models adjusted for fetal sex, maternal age (continuous), pre-pregnancy weight (continuous), height (continuous), education level (categorical), ethnicity (categorical), parity (categorical), smoking (categorical), alcohol use (categorical), folic acid use (categorical), and gestational age at ultrasound or delivery (continuous). Results represent associations from linear models of associations between pregnancy average organophosphate pesticide metabolite concentrations and outcomes from middle pregnancy, late pregnancy, and delivery in separate models. Abbreviations: DMPs, dimethyl phosphates; DEPs, diethyl phosphates; DAPs, dialkyl phosphates; CI, confidence interval.

Table S4. Adjusted[a] cross-sectional associations between urinary organophosphate pesticide metabolite concentrations measured in early pregnancy (<18 weeks of gestation) and standard deviation scores of fetal growth parameters measured during pregnancy by ultrasound and at delivery.

	Total DMPs	Total DEPs	Total DAPs
	Difference in SDS score (95% CI)	Difference in SDS score (95% CI)	Difference in SDS score (95% CI)
Head circumference			
Middle pregnancy	-0.09 (-0.28, 0.10)	-0.06 (-0.20, 0.08)	-0.10 (-0.30, 0.10)
Late pregnancy	-0.04 (-0.23, 0.15)	0.04 (-0.10, 0.18)	-0.03 (-0.23, 0.17)
Delivery	0.14 (-0.19, 0.47)	0.16 (-0.08, 0.40)	0.18 (-0.17, 0.52)
Length			
Middle pregnancy	-0.23 (-0.42, -0.04)	-0.21 (-0.34, -0.07)	-0.29 (-0.49, -0.10)
Late pregnancy	-0.10 (-0.29, 0.08)	-0.13 (-0.26, 0.01)	-0.16 (-0.36, 0.03)
Delivery	0.09 (-0.18, 0.36)	-0.02 (-0.21, 0.16)	0.10 (-0.17, 0.37)
Weight			
Middle pregnancy	-0.18 (-0.36, -0.01)	-0.14 (-0.26, -0.01)	-0.22 (-0.40, -0.03)
Late pregnancy	-0.12 (-0.31, 0.06)	-0.19 (-0.32, -0.05)	-0.19 (-0.38, 0.00)
Delivery	-0.03 (-0.22, 0.17)	-0.10 (-0.25, 0.04)	-0.05 (-0.25, 0.15)

a Models adjusted for fetal sex, maternal age (continuous), pre-pregnancy weight (continuous), height (continuous), education level (categorical), ethnicity (categorical), parity (categorical), smoking (categorical), alcohol use (categorical), folic acid use (categorical), and gestational age at ultrasound or delivery (continuous). Results represent associations from linear models of associations between pregnancy average organophosphate pesticide metabolite concentrations and outcomes from middle pregnancy, late pregnancy, and delivery in separate models. Abbreviations: DMPs, dimethyl phosphates; DEPs, diethyl phosphates; DAPs, dialkyl phosphates; CI, confidence interval.

Table S5. Adjusted[a] cross-sectional associations between urinary organophosphate pesticide metabolite concentrations measured in middle pregnancy (18-25 weeks of gestation) and standard deviation scores of fetal growth parameters measured during pregnancy by ultrasound and at delivery.

	Total DMPs	Total DEPs	Total DAPs
	Difference in SDS score (95% CI)	Difference in SDS score (95% CI)	Difference in SDS score (95% CI)
Head circumference			
Middle pregnancy	0.01 (-0.20, 0.22)	0.00 (-0.15, 0.14)	0.01 (-0.21, 0.23)
Late pregnancy	0.07 (-0.14, 0.27)	0.01 (-0.13, 0.15)	0.09 (-0.13, 0.30)
Delivery	0.34 (-0.09, 0.77)	0.07 (-0.15, 0.30)	0.31 (-0.13, 0.76)
Length			
Middle pregnancy	-0.15 (-0.35, 0.06)	-0.17 (-0.31, -0.04)	-0.20 (-0.41, 0.02)
Late pregnancy	0.01 (-0.19, 0.22)	-0.09 (-0.23, 0.05)	0.00 (-0.22, 0.21)
Delivery	0.09 (-0.18, 0.37)	0.05 (-0.14, 0.23)	0.08 (-0.20, 0.37)
Weight			
Middle pregnancy	-0.09 (-0.28, 0.10)	-0.18 (-0.31, -0.05)	-0.14 (-0.33, 0.06)
Late pregnancy	-0.02 (-0.22, 0.18)	-0.09 (-0.22, 0.05)	-0.01 (-0.22, 0.20)
Delivery	0.07 (-0.14, 0.28)	0.02 (-0.12, 0.16)	0.06 (-0.16, 0.28)

a Models adjusted for fetal sex, maternal age (continuous), pre-pregnancy weight (continuous), height (continuous), education level (categorical), ethnicity (categorical), parity (categorical), smoking (categorical), alcohol use (categorical), folic acid use (categorical), and gestational age at ultrasound or delivery (continuous). Results represent associations from linear models of associations between pregnancy average organophosphate pesticide metabolite concentrations and outcomes from middle pregnancy, late pregnancy, and delivery in separate models. Abbreviations: DMPs, dimethyl phosphates; DEPs, diethyl phosphates; DAPs, dialkyl phosphates; CI, confidence interval.

Table S6. Adjusted[a] cross-sectional associations urinary organophosphate pesticide metabolite concentrations measured in late pregnancy (>25 weeks of gestation) and standard deviation scores of fetal growth parameters measured during pregnancy by ultrasound and at delivery.

	Total DMPs	Total DEPs	Total DAPs
	Difference in SDS score (95% CI)	Difference in SDS score (95% CI)	Difference in SDS score (95% CI)
Head circumference			
Late pregnancy	0.00 (-0.20, 0.19)	0.05 (-0.09, 0.19)	0.01 (-0.20, 0.21)
Delivery	0.16 (-0.16, 0.48)	0.04 (-0.23, 0.32)	0.15 (-0.19, 0.48)
Length			
Late pregnancy	-0.09 (-0.29, 0.10)	-0.04 (-0.18, 0.11)	-0.09 (-0.30, 0.11)
Delivery	0.09 (-0.19, 0.37)	-0.09 (-0.30, 0.13)	0.05 (-0.24, 0.34)
Weight			
Late pregnancy	-0.05 (-0.24, 0.14)	0.03 (-0.11, 0.16)	-0.04 (-0.24, 0.16)
Delivery	-0.01 (-0.21, 0.19)	0.04 (-0.10, 0.19)	0.00 (-0.22, 0.21)

a Models adjusted for fetal sex, maternal age (continuous), pre-pregnancy weight (continuous), height (continuous), education level (categorical), ethnicity (categorical), parity (categorical), smoking (categorical), alcohol use (categorical), folic acid use (categorical), and gestational age at ultrasound or delivery (continuous). Results represent associations from linear models of associations between pregnancy average organophosphate pesticide metabolite concentrations and outcomes from middle pregnancy, late pregnancy, and delivery in separate models. Abbreviations: DMPs, dimethyl phosphates; DEPs, diethyl phosphates; DAPs, dialkyl phosphates; CI, confidence interval.

Table S7a. Adjusted[a] repeated measures associations between pregnancy average urinary dialkyl phosphate pesticide metabolite concentrations and standard deviation scores of fetal growth parameters measured during pregnancy by ultrasound and at delivery in models stratified by *PON1*$_{108}$ genotype.

	TT		CT		CC	
	Difference in SDS score (95% CI)		Difference in SDS score (95% CI)		Difference in SDS score (95% CI)	
Head circumference						
Middle pregnancy	0.10	(-0.76, 0.96)	0.03	(-0.52, 0.58)	0.03	(-0.52, 0.58)
Late pregnancy	0.31	(-0.55, 1.16)	0.13	(-0.26, 0.52)	-0.03	(-0.50, 0.43)
Delivery	0.51	(-0.69, 1.71)	0.23	(-0.42, 0.88)	0.34	(-0.28, 0.97)
p (interaction)[b]						0.76
Length						
Middle pregnancy	-0.35	(-1.22, 0.53)	-0.54	(-1.04, -0.04)	-0.73	(-1.35, -0.11)
Late pregnancy	0.07	(-0.63, 0.78)	-0.26	(-0.63, 0.12)	-0.19	(-0.66, 0.29)
Delivery	0.50	(-0.59, 1.59)	0.03	(-0.47, 0.53)	0.36	(-0.28, 1.00)
p (interaction)[b]						0.62
Weight						
Middle pregnancy	-0.17	(-1.00, 0.67)	-0.25	(-0.72, 0.23)	-0.51	(-1.11, 0.09)
Late pregnancy	-0.01	(-0.66, 0.64)	-0.13	(-0.51, 0.24)	-0.24	(-0.70, 0.22)
Delivery	0.15	(-0.73, 1.03)	-0.02	(-0.50, 0.46)	0.03	(-0.50, 0.56)
p (interaction)[b]						0.86

a Models adjusted for fetal sex, maternal age (continuous), pre-pregnancy weight (continuous), height (continuous), education level (categorical), ethnicity (categorical), parity (categorical), smoking (categorical), alcohol use (categorical), folic acid use (categorical), and gestational age at ultrasound or delivery (continuous). Models contain an interaction term between exposure concentration and gestational age at ultrasound/delivery, a random intercept for each participant, and a random slope for gestational age at ultrasound/delivery. Models of head circumference, femur length, and weight include measurements taken at delivery (head circumference, birth length, and birth weight, respectively). [b]p values for the interaction term between exposure concentration and genotype. Abbreviations: CI, confidence interval; SDS, standard deviation score.

Table S7b. Adjusted[a] repeated measures associations between pregnancy average urinary dialkyl phosphate pesticide metabolite concentrations and standard deviation scores of fetal growth parameters measured during pregnancy by ultrasound and at delivery in models stratified by PON1-161 genotype

	GG		CG		CC	
	Difference in SDS score (95% CI)		Difference in SDS score (95% CI)		Difference in SDS score (95% CI)	
Head circumference						
Middle pregnancy	-0.12	(-1.59, 1.35)	-0.37	(-0.93, 0.20)	0.06	(-0.42, 0.55)
Late pregnancy	0.02	(-1.14, 1.18)	-0.04	(-0.52, 0.43)	0.22	(-0.13, 0.57)
Delivery	0.16	(-1.39, 1.71)	0.28	(-0.46, 1.03)	0.37	(-0.16, 0.90)
p (interaction)[b]						0.47
Length						
Middle pregnancy	-0.92	(-2.36, 0.53)	-1.01	(-1.61, -0.40)	-0.23	(-0.67, 0.22)
Late pregnancy	-0.56	(-1.66, 0.54)	-0.28	(-0.71, 0.15)	-0.03	(-0.38, 0.32)
Delivery	-0.20	(-1.58, 1.17)	0.44	(-0.15, 1.03)	0.17	(-0.32, 0.66)
p (interaction)[b]						0.18
Weight						
Middle pregnancy	-0.36	(-1.62, 0.89)	-0.67	(-1.19, -0.15)	-0.05	(-0.47, 0.37)
Late pregnancy	-0.24	(-1.31, 0.84)	-0.30	(-0.75, 0.15)	0.01	(-0.32, 0.35)
Delivery	-0.11	(-1.39, 1.17)	0.08	(-0.51, 0.67)	0.08	(-0.35, 0.51)
p (interaction)[b]						0.19

a Models adjusted for fetal sex, maternal age (continuous), pre-pregnancy weight (continuous), height (continuous), education level (categorical), ethnicity (categorical), parity (categorical), smoking (categorical), alcohol use (categorical), folic acid use (categorical), and gestational age at ultrasound or delivery (continuous). Models contain an interaction term between exposure concentration and gestational age at ultrasound/delivery, a random intercept for each participant, and a random slope for gestational age at ultrasound/delivery. Models of head circumference, femur length, and weight include measurements taken at delivery (head circumference, birth length, and birth weight, respectively). bp values for the interaction term between exposure concentration and genotype. Abbreviations: CI, confidence interval; SDS, standard deviation score..

Table S7c. Adjusted[a] repeated measures associations between pregnancy average urinary dialkyl phosphate pesticide metabolite concentrations and standard deviation scores of fetal growth parameters measured during pregnancy by ultrasound and at delivery in models stratified by PON1-L55m genotype.

	TT		AT		AA	
	Difference in SDS score (95% CI)		Difference in SDS score (95% CI)		Difference in SDS score (95% CI)	
Head circumference						
Middle pregnancy	-0.03	(-1.05, 1.00)	-0.43	(-1.02, 0.16)	0.14	(-0.39, 0.67)
Late pregnancy	0.46	(-0.36, 1.29)	-0.03	(-0.50, 0.43)	0.14	(-0.27, 0.55)
Delivery	0.95	(-0.24, 2.13)	0.36	(-0.33, 1.06)	0.14	(-0.52, 0.80)
p (interaction)[b]						0.80
Length						
Middle pregnancy	-0.81	(-1.97, 0.35)	-0.54	(-1.10, 0.03)	-0.52	(-1.05, 0.00)
Late pregnancy	-0.32	(-1.15, 0.51)	-0.09	(-0.53, 0.35)	-0.19	(-0.57, 0.20)
Delivery	0.24	(-0.93, 1.42)	0.36	(-0.21, 0.93)	0.15	(-0.38, 0.68)
p (interaction)[b]						0.93
Weight						
Middle pregnancy	-0.54	(-1.40, 0.32)	-0.46	(-0.98, 0.06)	-0.13	(-0.62, 0.35)
Late pregnancy	-0.22	(-0.91, 0.46)	-0.25	(-0.71, 0.20)	-0.01	(-0.40, 0.38)
Delivery	0.09	(-0.83, 1.01)	-0.04	(-0.66, 0.57)	0.11	(-0.40, 0.62)
p (interaction)[b]						0.39

a Models adjusted for fetal sex, maternal age (continuous), pre-pregnancy weight (continuous), height (continuous), education level (categorical), ethnicity (categorical), parity (categorical), smoking (categorical), alcohol use (categorical), folic acid use (categorical), and gestational age at ultrasound or delivery (continuous). Models contain an interaction term between exposure concentration and gestational age at ultrasound/delivery, a random intercept for each participant, and a random slope for gestational age at ultrasound/delivery. Models of head circumference, femur length, and weight include measurements taken at delivery (head circumference, birth length, and birth weight, respectively). bp values for the interaction term between exposure concentration and genotype. Abbreviations: CI, confidence interval; SDS, standard deviation score. Note: Model for femur length under PON1-L55 would not converge and thus results presented are for model without inclusion of a random slope for gestational age at ultrasound/delivery.

Table S7d. Adjusted[a] repeated measures associations between pregnancy average urinary dialkyl phosphate pesticide metabolite concentrations and standard deviation scores of fetal growth parameters measured during pregnancy by ultrasound and at delivery in models stratified by PON1-909 genotype.

	GG		CG		CC	
	Difference in SDS score (95% CI)		Difference in SDS score (95% CI)		Difference in SDS score (95% CI)	
Head circumference						
Middle pregnancy	0.22	(-0.72, 1.16)	-0.02	(-0.56, 0.52)	-0.36	(-0.99, 0.27)
Late pregnancy	0.41	(-0.25, 1.08)	0.08	(-0.39, 0.54)	0.04	(-0.49, 0.58)
Delivery	0.61	(-0.38, 1.59)	0.17	(-0.50, 0.84)	0.45	(-0.35, 1.24)
p (interaction)[b]						0.58
Length						
Middle pregnancy	-0.28	(-1.07, 0.51)	-0.67	(-1.15, -0.19)	-0.55	(-1.16, 0.06)
Late pregnancy	0.22	(-0.46, 0.91)	-0.33	(-0.73, 0.06)	-0.12	(-0.57, 0.33)
Delivery	0.73	(-0.27, 1.72)	0.00	(-0.54, 0.54)	0.32	(-0.35, 0.98)
p (interaction)[b]						0.39
Weight						
Middle pregnancy	-0.14	(-0.86, 0.59)	-0.35	(-0.78, 0.07)	-0.33	(-0.89, 0.22)
Late pregnancy	0.08	(-0.50, 0.67)	-0.23	(-0.61, 0.16)	-0.10	(-0.58, 0.39)
Delivery	0.30	(-0.53, 1.13)	-0.10	(-0.65, 0.45)	0.13	(-0.50, 0.77)
p (interaction)[b]						0.74

a Models adjusted for fetal sex, maternal age (continuous), pre-pregnancy weight (continuous), height (continuous), education level (categorical), ethnicity (categorical), parity (categorical), smoking (categorical), alcohol use (categorical), folic acid use (categorical), and gestational age at ultrasound or delivery (continuous). Models contain an interaction term between exposure concentration and gestational age at ultrasound/delivery, a random intercept for each participant, and a random slope for gestational age at ultrasound/delivery. Models of head circumference, femur length, and weight include measurements taken at delivery (head circumference, birth length, and birth weight, respectively). bp values for the interaction term between exposure concentration and genotype. Abbreviations: CI, confidence interval; SDS, standard deviation score.

Table S7e. Adjusted repeated measures associations between pregnancy average urinary dialkyl phosphate pesticide metabolite concentrations and standard deviation scores of fetal growth parameters measured during pregnancy by ultrasound and at delivery in models stratified by PON1 Q192R genotype.

	TT (QQ)		CT (QR)		CC (RR)	
	Difference in SDS score (95% CI)		Difference in SDS score (95% CI)		Difference in SDS score (95% CI)	
Head circumference						
Middle pregnancy	-0.35	(-0.89, 0.18)	-0.05	(-0.59, 0.49)	0.66	(-0.62, 1.95)
Late pregnancy	0.09	(-0.37, 0.55)	-0.03	(-0.44, 0.39)	0.86	(-0.19, 1.91)
Delivery	0.53	(-0.21, 1.27)	-0.01	(-0.56, 0.55)	1.05	(-0.26, 2.37)
p (interaction)[b]						0.20
Length						
Middle pregnancy	-0.69	(-1.24, -0.13)	-0.42	(-0.92, 0.09)	-0.63	(-2.12, 0.86)
Late pregnancy	-0.13	(-0.54, 0.28)	-0.18	(-0.55, 0.19)	-0.07	(-1.16, 1.02)
Delivery	0.43	(-0.18, 1.03)	0.06	(-0.45, 0.57)	0.49	(-1.15, 2.13)
p (interaction)[b]						0.90
Weight						
Middle pregnancy	-0.47	(-0.95, 0.01)	-0.20	(-0.68, 0.28)	-0.30	(-1.57, 0.97)
Late pregnancy	-0.18	(-0.59, 0.24)	-0.20	(-0.57, 0.16)	0.27	(-0.80, 1.34)
Delivery	0.11	(-0.43, 0.65)	-0.21	(-0.66, 0.24)	0.84	(-0.75, 2.43)
p (interaction)[b]						0.35

a Models adjusted for fetal sex, maternal age (continuous), pre-pregnancy weight (continuous), height (continuous), education level (categorical), ethnicity (categorical), parity (categorical), smoking (categorical), alcohol use (categorical), folic acid use (categorical), and gestational age at ultrasound or delivery (continuous). Models contain an interaction term between exposure concentration and gestational age at ultrasound/delivery, a random intercept for each participant, and a random slope for gestational age at ultrasound/delivery. Models of head circumference, femur length, and weight include measurements taken at delivery (head circumference, birth length, and birth weight, respectively). bp values for the interaction term between exposure concentration and genotype. Abbreviations: CI, confidence interval; SDS, standard deviation score.

Table S8. Unadjusted[a] repeated measures associations between pregnancy average urinary organophosphate pesticide metabolite concentrations and standard deviation scores of fetal growth parameters measured during pregnancy by ultrasound and at delivery.

	Total DMPs		Total DEPs		Total DAPs	
	Difference in SDS score (95% CI)		Difference in SDS score (95% CI)		Difference in SDS score (95% CI)	
Head circumference						
Middle pregnancy	-0.13	(-0.45, 0.20)	0.00	(-0.15, 0.15)	-0.10	(-0.44, 0.23)
Late pregnancy	0.11	(-0.14, 0.35)	0.06	(-0.05, 0.18)	0.11	(-0.14, 0.37)
Delivery	0.34	(-0.06, 0.75)	0.13	(-0.06, 0.32)	0.33	(-0.10, 0.76)
Length						
Middle pregnancy	-0.57	(-0.87, -0.28)	-0.20	(-0.34, -0.06)	-0.65	(-0.96, -0.35)
Late pregnancy	-0.20	(-0.42, 0.02)	-0.09	(-0.20, 0.01)	-0.25	(-0.48, -0.03)
Delivery	0.17	(-0.15, 0.50)	0.02	(-0.14, 0.17)	0.15	(-0.18, 0.48)
Weight						
Middle pregnancy	-0.36	(-0.63, -0.09)	-0.18	(-0.30, -0.06)	-0.41	(-0.68, -0.13)
Late pregnancy	-0.19	(-0.40, 0.02)	-0.10	(-0.20, 0.00)	-0.22	(-0.44, 0.00)
Delivery	-0.02	(-0.30, 0.26)	-0.02	(-0.15, 0.11)	-0.04	(-0.33, 0.25)

a Models contain an interaction term between exposure concentration and gestational age at ultrasound/delivery, a random intercept for each participant, and a random slope for gestational age at ultrasound/delivery. Abbreviations: DMPs, dimethyl phosphates; DEPs, diethyl phosphates; DAPs, dialkyl phosphates; CI, confidence interval.

Table S9. Adjusted[a] repeated measures associations between pregnancy average urinary organophosphate pesticide metabolite concentrations and standard deviation scores of fetal growth parameters measured during pregnancy by ultrasound and at delivery in dataset without outcome imputed.

	Total DMPs		Total DEPs		Total DAPs	
	Difference in SDS score (95% CI)		Difference in SDS score (95% CI)		Difference in SDS score (95% CI)	
Head circumference						
Middle pregnancy	-0.03	(-0.35, 0.30)	0.02	(-0.13, 0.16)	-0.01	(-0.34, 0.33)
Late pregnancy	0.01	(-0.23, 0.25)	0.05	(-0.06, 0.16)	0.02	(-0.23, 0.27)
Delivery	0.04	(-0.35, 0.44)	0.09	(-0.10, 0.28)	0.05	(-0.35, 0.44)
Length						
Middle pregnancy	-0.46	(-0.78, -0.15)	-0.18	(-0.32, -0.04)	-0.54	(-0.86, -0.22)
Late pregnancy	-0.17	(-0.40, 0.06)	-0.08	(-0.18, 0.02)	-0.21	(-0.44, 0.02)
Delivery	0.12	(-0.23, 0.47)	0.02	(-0.13, 0.17)	0.12	(-0.24, 0.48)
Weight						
Middle pregnancy	-0.29	(-0.57, -0.02)	-0.17	(-0.29, -0.05)	-0.34	(-0.62, -0.06)
Late pregnancy	-0.12	(-0.34, 0.09)	-0.09	(-0.19, 0.01)	-0.16	(-0.38, 0.07)
Delivery	0.05	(-0.23, 0.32)	-0.01	(-0.14, 0.11)	0.03	(-0.26, 0.31)

a Models adjusted for fetal sex, maternal age (continuous), pre-pregnancy weight (continuous), height (continuous), education level (categorical), ethnicity (categorical), parity (categorical), smoking (categorical), alcohol use (categorical), folic acid use (categorical), and gestational age at ultrasound or delivery (continuous). Models contain an interaction term between exposure concentration and gestational age at ultrasound/delivery, a random intercept for each participant, and a random slope for gestational age at ultrasound/delivery. Abbreviations: DMPs, dimethyl phosphates; DEPs, diethyl phosphates; DAPs, dialkyl phosphates; CI, confidence interval. N=784 for all models since each participant had at least one outcome measurement; however, the number of observations at each time point differed as follows: head circumference (n=774 for middle pregnancy; n=777 for late pregnancy; n=478 for delivery); length (n=779 for middle pregnancy; n=784 for late pregnancy; n=562 for delivery); weight (n=777 for middle pregnancy; n=782 for late pregnancy; n=784 for delivery).

Table S10. Adjusted[a] repeated measures associations between mutually adjusted pregnancy average urinary dimethyl phosphate and diethyl phosphate pesticide metabolite concentrations and standard deviation scores of fetal growth parameters measured during pregnancy by ultrasound and at delivery.

	Total DMPs		Total DEPs	
	Difference in SDS score (95% CI)		Difference in SDS score (95% CI)	
Head circumference				
Middle pregnancy	-0.17	(-0.51, 0.17)	-0.01	(-0.16, 0.14)
Late pregnancy	0.06	(-0.20, 0.32)	0.06	(-0.06, 0.17)
Delivery	0.29	(-0.12, 0.71)	0.12	(-0.07, 0.31)
Length				
Middle pregnancy	-0.45	(-0.77, -0.14)	-0.17	(-0.31, -0.03)
Late pregnancy	-0.08	(-0.32, 0.16)	-0.06	(-0.17, 0.05)
Delivery	0.29	(-0.04, 0.63)	0.05	(-0.11, 0.21)
Weight				
Middle pregnancy	-0.23	(-0.51, 0.06)	-0.16	(-0.29, -0.03)
Late pregnancy	-0.06	(-0.29, 0.17)	-0.08	(-0.18, 0.02)
Delivery	-0.15	(-0.93, 0.64)	0.00	(-0.13, 0.13)

a Models adjusted for fetal sex, maternal age (continuous), pre-pregnancy weight (continuous), height (continuous), education level (categorical), ethnicity (categorical), parity (categorical), smoking (categorical), alcohol use (categorical), folic acid use (categorical), and gestational age at ultrasound or delivery (continuous). Models contain an interaction term between exposure concentration and gestational age at ultrasound/delivery, a random intercept for each participant, and a random slope for gestational age at ultrasound/delivery. Abbreviations: DMPs, dimethyl phosphates; DEPs, diethyl phosphates; CI, confidence interval.

Table S11. Adjusted[a] repeated measures associations between pregnancy average urinary dialkyl phosphate concentrations and standard deviation scores of fetal growth parameters measured during pregnancy by ultrasound and at delivery with the application of regression calibration.

	Primary model[b]	Regression calibration[b]
	Difference in SDS score (95% CI)	Difference in SDS score (95% CI)
Head circumference		
Middle pregnancy	-0.15 (-0.47, 0.17)	-0.54 (-1.72, 0.64)
Late pregnancy	0.07 (-0.17, 0.30)	0.26 (-0.61, 1.24)
Delivery	0.28 (-0.04, 0.61)	1.05 (-0.14, 2.45)
Length		
Middle pregnancy	-0.57 (-0.88, -0.26)	-2.11 (-3.24, -0.97)
Late pregnancy	-0.17 (-0.40, 0.06)	-0.64 (-1.48, 0.21)
Delivery	0.22 (-0.09, 0.54)	0.83 (-0.32, 1.99)
Weight		
Middle pregnancy	-0.33 (-0.61, -0.05)	-1.22 (-2.26, -0.19)
Late pregnancy	-0.15 (-0.37, 0.07)	-0.56 (-1.37, 0.25)
Delivery	0.03 (-0.25, 0.31)	-0.11 (-0.93, 1.15)

a Models adjusted for fetal sex, maternal age (continuous), pre-pregnancy weight (continuous), height (continuous), education level (categorical), ethnicity (categorical), parity (categorical), smoking (categorical), alcohol use (categorical), folic acid use (categorical), and gestational age at ultrasound or delivery (continuous). Models contain an interaction term between exposure concentration and gestational age at ultrasound/delivery, a random intercept for each participant, and a random slope for gestational age at ultrasound/delivery.
b Results from 1 imputed dataset and thus differ slightly from results in Table 4.
Abbreviation: CI, confidence interval.

Table S12. Adjusted[a] repeated measures associations between pregnancy average urinary dialkyl phosphate concentrations and standard deviation scores of fetal growth parameters measured during pregnancy by ultrasound and at delivery with marital status and family income included as covariates.

		Total DAPs Difference in SDS score (95% CI)	
Head circumference			
	Middle pregnancy	-0.15	(-0.48, 0.19)
	Late pregnancy	0.07	(-0.19, 0.33)
	Delivery	0.28	(-0.15, 0.72)
Length			
	Middle pregnancy	-0.58	(-0.89, -0.26)
	Late pregnancy	-0.18	(-0.41, 0.06)
	Delivery	0.22	(-0.11, 0.56)
Weight			
	Middle pregnancy	-0.35	(-0.64, -0.07)
	Late pregnancy	-0.17	(-0.40, 0.05)
	Delivery	0.01	(-0.28, 0.29)

a Models adjusted for fetal sex, maternal age (continuous), pre-pregnancy weight (continuous), height (continuous), education level (categorical), ethnicity (categorical), parity (categorical), smoking (categorical), alcohol use (categorical), folic acid use (categorical), gestational age at ultrasound or delivery (continuous), marital status (categorical), and household income (categorical). Models contain an interaction term between exposure concentration and gestational age at ultrasound/delivery, a random intercept for each participant, and a random slope for gestational age at ultrasound/delivery. Abbreviations: DMPs, dimethyl phosphates; DEPs, diethyl phosphates; DAPs, dialkyl phosphates; CI, confidence interval.

Table S13. Adjusted[a] cross-sectional associations between early pregnancy urinary dialkyl phosphate concentrations and standard deviation scores of fetal growth parameters measured during pregnancy by ultrasound and at delivery with season of sample collection included as a covariate.

		Total DAPs Difference in SDS score (95% CI)	
Head circumference			
	Middle pregnancy	-0.10	(-0.30, 0.10)
	Late pregnancy	-0.03	(-0.23, 0.17)
	Delivery	0.14	(-0.29, 0.57)
Length			
	Middle pregnancy	-0.30	(-0.49, -0.10)
	Late pregnancy	-0.15	(-0.35, 0.05)
	Delivery	0.05	(-0.21, 0.32)
Weight			
	Middle pregnancy	-0.22	(-0.41, -0.03)
	Late pregnancy	-0.18	(-0.37, 0.01)
	Delivery	-0.05	(-0.25, 0.15)

a Models adjusted for fetal sex, maternal age (continuous), pre-pregnancy weight (continuous), height (continuous), education level (categorical), ethnicity (categorical), parity (categorical), smoking (categorical), alcohol use (categorical), folic acid use (categorical), gestational age at ultrasound or delivery (continuous), and season of early pregnancy urine sample collection. Abbreviations: DMPs, dimethyl phosphates; DEPs, diethyl phosphates; DAPs, dialkyl phosphates; CI, confidence interval.

Table S14. Adjusted[a] repeated measures associations between pregnancy average urinary dialkyl phosphate concentrations and standard deviation scores of fetal growth parameters measured during pregnancy by ultrasound and at delivery among term births (>=37 weeks) only.

		Total DAPs Difference in SDS score (95% CI)	
Head circumference			
	Middle pregnancy	-0.15	(-0.49, 0.19)
	Late pregnancy	0.05	(-0.23, 0.32)
	Delivery	0.24	(-0.26, 0.75)
Length			
	Middle pregnancy	-0.62	(-0.94, -0.30)
	Late pregnancy	-0.23	(-0.47, 0.00)
	Delivery	0.16	(-0.17, 0.49)
Weight			
	Middle pregnancy	-0.37	(-0.66, -0.09)
	Late pregnancy	-0.21	(-0.43, 0.02)
	Delivery	-0.04	(-0.32, 0.25)

a Models adjusted for fetal sex, maternal age (continuous), pre-pregnancy weight (continuous), height (continuous), education level (categorical), ethnicity (categorical), parity (categorical), smoking (categorical), alcohol use (categorical), folic acid use (categorical), gestational age at ultrasound or delivery (continuous), marital status (categorical), and household income (categorical). Models contain an interaction term between exposure concentration and gestational age at ultrasound/delivery, a random intercept for each participant, and a random slope for gestational age at ultrasound/delivery. Abbreviations: DMPs, dimethyl phosphates; DEPs, diethyl phosphates; DAPs, dialkyl phosphates; CI, confidence interval.

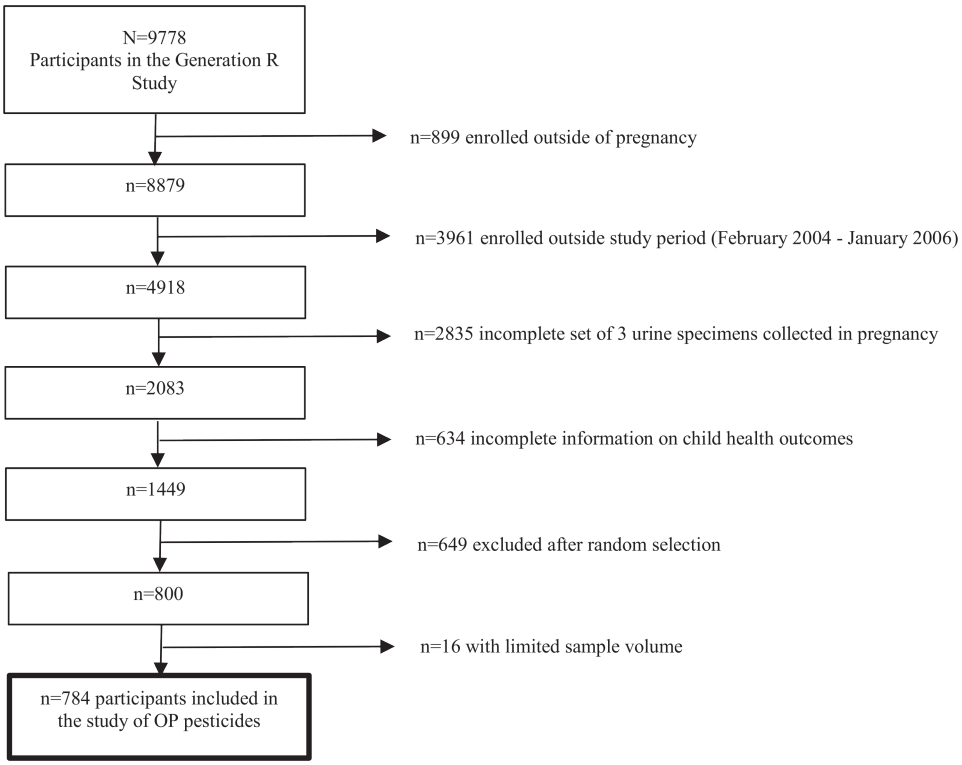

Figure S1. Flow chart from the overall Generation R study population to the participants included in the present study.

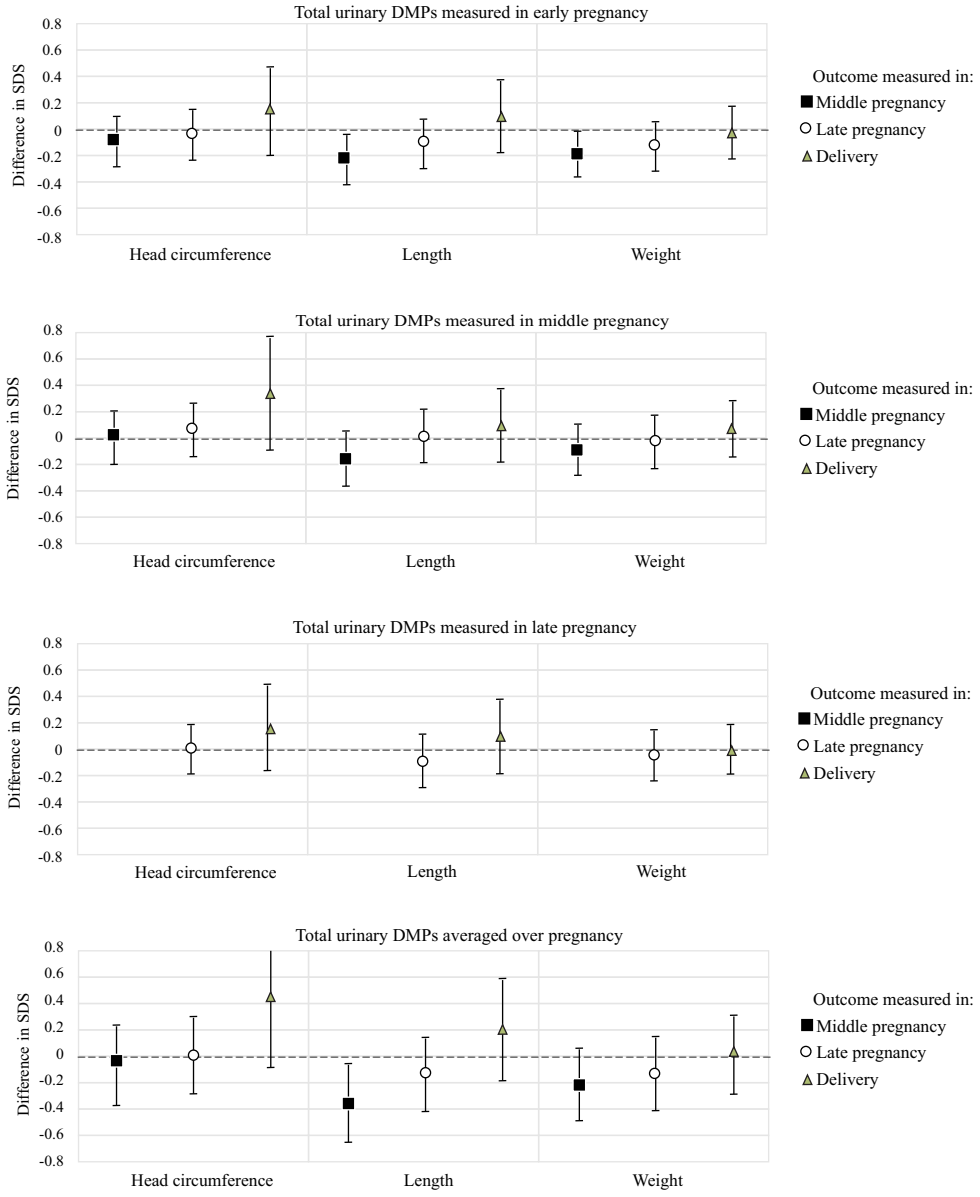

Figure S2. Adjusted cross-sectional associations between visit-specific total dimethyl phosphate (DMP) concentrations and standard deviation scores of fetal growth parameters measured during pregnancy by ultrasound and by clinical examination at delivery. Model adjusted for fetal sex, maternal age (continuous), pre-pregnancy weight (continuous), height (continuous), education level (categorical), maternal ethnicity (categorical), parity (categorical), smoking (categorical), alcohol use (categorical), folic acid use (categorical), and gestational age at ultrasound or delivery (continuous). Abbreviations: DAPs, dialkyl phosphates; CI, confidence interval.

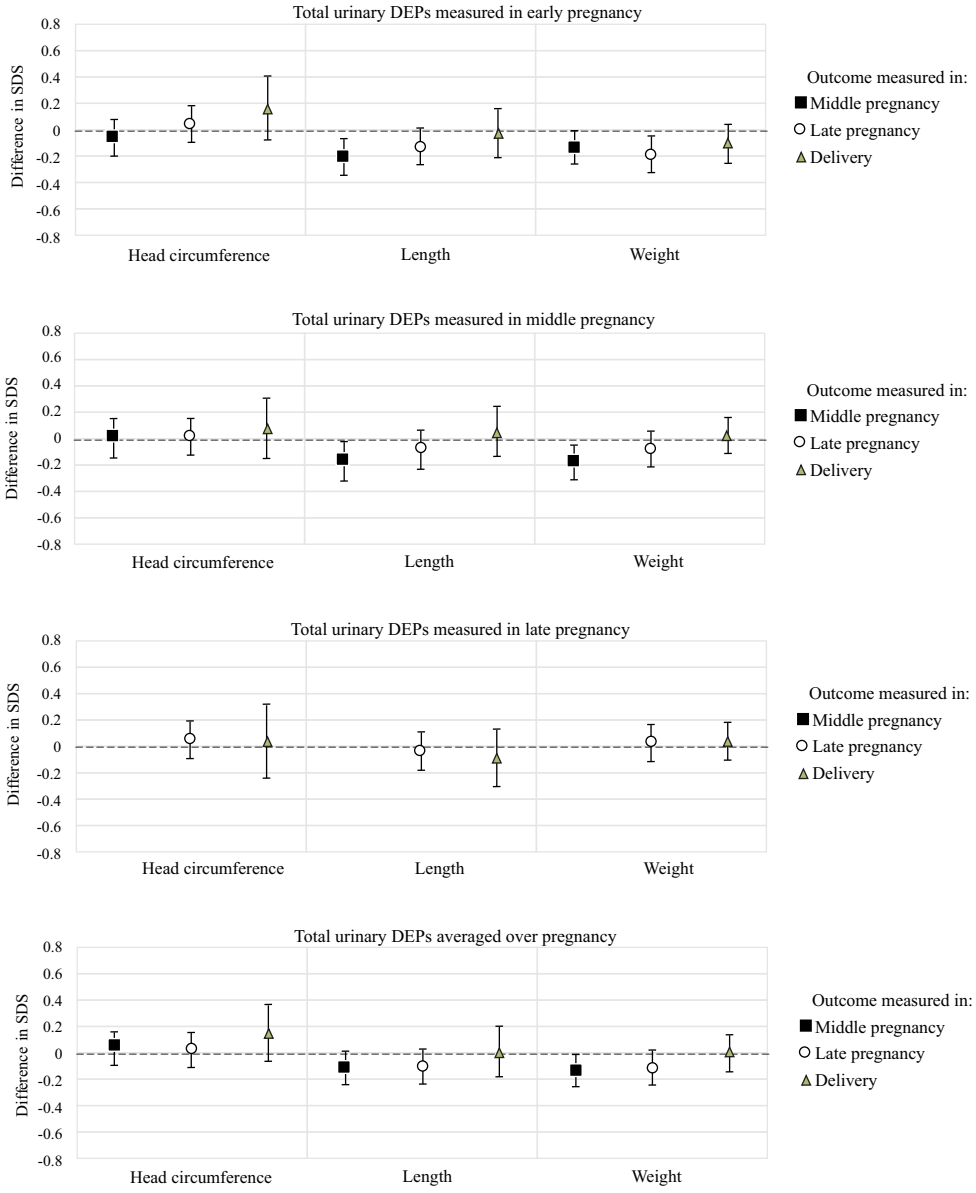

Figure S3. Adjusted cross-sectional associations between visit-specific total diethyl phosphate (DEP) concentrations and standard deviation scores of fetal growth parameters measured during pregnancy by ultrasound and by clinical examination at delivery. Model adjusted for fetal sex, maternal age (continuous), pre-pregnancy weight (continuous), height (continuous), education level (categorical), maternal ethnicity (categorical), parity (categorical), smoking (categorical), alcohol use (categorical), folic acid use (categorical), and gestational age at ultrasound or delivery (continuous). Abbreviations: DAPs, dialkyl phosphates; CI, confidence interval.

Part V

11

Chapter 11

General discussion

General Discussion

Women and men are universally exposed to a broad range of chemicals present in consumer products.[1,2] Phthalates and bisphenols are synthetic compounds which exist in numerous products such as cosmetics and food packaging materials.[1,3,4] Organophosphate (OP) pesticides are insecticides that are commonly used for crop protection, and exposure occurs readily through the diet.[3-5] Because these non-persistent chemicals may occur in many different combinations and concentrations in a variety of products, all men and women, including pregnant women, are exposed to a complex chemical mixture. The fetal brain can be exposed to these chemicals as they have the ability to surpass the placenta and blood–brain barrier.[6,7] Animal studies have linked low-dose exposure to these chemicals during pregnancy to adverse effects on the neurodevelopment of the offspring. In some cases, the few epidemiological studies investigating fetal exposure to these chemical groups are suggestive of an effect on neurodevelopment but are overall inconclusive.

The present thesis examined the relationship between prenatal exposure to phthalates, bisphenols, and OP pesticides and neurodevelopment in children by studying (i) the determinants of exposure to non-persistent chemicals during pregnancy, (ii) exploring the association of prenatal exposure to non-persistent chemicals with neurodevelopment in children, and (iii) investigating the effect of exposure to these non-persistent chemicals on the potential mediators—such as thyroid function, brain structure, and fetal growth—of the association with neurodevelopment. These aims were explored using data from the Generation R Study, that is advantageous by having a large sample size; detailed follow-up information of prenatal and postnatal development of the fetus and child, respectively; multiple measurements (early, mid-, and late pregnancy) of exposure to non-persistent chemicals across gestation; and availability of detailed demographic information. In this general discussion, the main findings per chapter are presented in brief before discussing the methodological considerations. Then, the implications of this research, future directions for the field, and the conclusion are presented.

Main findings

Determinants of exposure to non-persistent chemicals

The identification of the potential sources and routes of exposure to non-persistent chemicals is particularly useful for future health risk management strategies and intervention studies aimed at reducing exposure. Moreover, identifying potential determinants of exposure to non-persistent chemicals helps to understand which possible confounders may exist in the exposure-disease associations.

Phthalate and bisphenol concentrations were frequently determined in maternal urinary samples. The concentrations observed in the Generation R Study participants were similar to those observed in other Western studies from the same time period.[8] However, OP pesticide concentrations were 2–3 fold higher than those in most other studies previously conducted (Chapter 2). The relatively high OP pesticide exposure in our study may be related to the high consumption of fruits and the intense farming practices in the Netherlands. The fruit and vegetable intake of our study sample in the Dutch population (median of 295 g/day) was higher as compared to that of nationally representative women of reproductive age from the United States (median of 167 g/day) where most other studies were conducted.[9,10] Furthermore, approximately 50% of the surface area of the Netherlands is used for agricultural purposes, and more pesticides and fertilizers per km^2 of farmland are applied compared with most other OECD countries such as the United States.[11] Whether dietary patterns and intense farming activities increase the level of OP pesticide exposure through the consumption of food products cannot be confirmed. However, between 1998 and 2008, approximately one-third of all insecticides used in the Netherlands were OP pesticides[12], and detectable OP pesticide residues were found on a large proportion of fruits and vegetables tested between 2004 and 2006 in the Netherlands.[13]

Lifestyle- and socioeconomic status (SES)-related factors were associated with phthalate, bisphenol, and OP pesticide exposure concentrations in pregnant women, and the direction of the effect differed per chemical class.[8] For example, a body mass index (BMI) of >30 kg/m^2 and no folic acid supplement intake were predictive of higher phthalate and bisphenol concentrations, and a lower educational level was predictive of higher phthalate and bisphenol concentrations. In contrast, protective lifestyle and higher SES characteristics, such as having a healthy BMI, being married, being a non-smoker during pregnancy, and having a high education level and high income, were associated with higher OP pesticide exposure levels. It is conceivable that these associations with lifestyle and SES factors are driven by their relationship with the use of consumer goods and a healthier diet. SES is associated with food-purchasing patterns[14,15] and higher SES is predictive of higher consumption of fruit and vegetables[16-18], an important source of OP pesticide exposure.[5] Moreover, pregnant women with low SES may consume more canned and packaged products, which results in higher phthalate and bisphenol exposure levels through contact with food packaging materials[19] because fresh products with a short expiration are often more expensive. Additionally, fat-rich diets are predictive of high concentrations of phthalates[20,21], which may explain why women with overweight had higher phthalate concentrations.

Furthermore, the intake of several dietary products was weakly associated with phthalate and bisphenol concentrations. For example, women who showed the highest 10% of soft

drink consumption had higher phthalate concentrations, but these associations were not observed after multiple testing correction. However, we found that higher dietary intake of fruits was a strong predictor of higher OP pesticide concentrations. This is consistent with the observation that OP pesticide residues have frequently been detected on and in fruits, which are, therefore, an important source of exposure.[5,13]

The present study provides useful insights into the potential determinants of exposure to non-persistent chemicals and may help to identify the potential sources and routes of exposure to non-persistent chemicals. However, the study could have been strengthened by the collection of data on factors such personal care product use, vinyl flooring in households, and the consumption of packaged versus non-packaged food items. Regarding OP pesticides, the exposure generally occurs through the consumption of food. However, information on the use of pest control items and the consumption of organic versus non-organic food items are such informative factors. These data would be useful in the detection of more potential sources and routes of exposure to non-persistent chemicals and potentially help in the development of targeted interventions aimed to reduce the exposure levels.

Taken all together, these findings demonstrate not only that non-persistent chemicals are frequently detected among pregnant women in the Netherlands but also that OP pesticide metabolite concentrations in this population were higher than those reported in most other birth cohort studies. Furthermore, this research shows that several lifestyle and SES factors are associated with exposure concentrations and that the direction of the association varies for the chemical classes. Moreover, this work strengthens the hypothesis that dietary intake is predictive of OP pesticide exposure among women living in an urban environment. The next subsections will describe the potential effects of prenatal exposure to phthalates, bisphenols, and OP pesticides on offspring neurodevelopment.

Exposure to non-persistent chemicals and neurodevelopment in children

As a second aim, we explored the association between prenatal exposure to non-persistent chemicals and neurodevelopment in children. Chapters 3 (phthalates and bisphenols) and 4 (OP pesticides) explored the association between prenatal exposure to non-persistent chemicals and cognition as measured by the Snijders-Oomen non-verbal intelligence test–revise score. Chapter 5 investigated whether OP pesticide exposure is associated with autistic traits and attention-deficit hyperactivity disorder (ADHD) in children. The results from this study make several important contributions to the existing body of literature on environmental exposure and neurodevelopment.

First, a 10-fold higher phthalate exposure during early pregnancy was consistently associated with 1.7–2.4 points lower child non-verbal IQ score. Fewer inverse associations were

observed in this study for exposure in late pregnancy and mean exposure across pregnancy. These results are important because all other previous studies assessing prenatal phthalate exposure during pregnancy and IQ have examined the exposure during mid- or late pregnancy and, therefore, may explain why these studies have reported diverse findings.[22-26]

Similarly, we found that only the late-pregnancy concentrations of the metabolite mono(3-carboxypropyl) phthalate were predictive of lower non-verbal IQ and observed no association between non-verbal IQ and other mid- and late-pregnancy phthalate metabolite concentrations. Although the critical window(s) of fetal neurotoxicity of phthalates is uncertain and future studies should confirm these findings, our findings propose that early pregnancy is a susceptible period.

Second, the present study is one of the first to explore the cognitive effects of prenatal exposure to bisphenol A and its substitutes bisphenol F and bisphenol S. Only one previous study has examined the effects of early-gestational exposure to a mixture of chemicals with endocrine disrupting properties such as bisphenols and identified bisphenol F as an important driver of the overall mixture effect on reduced IQ.[27] Industries exploring alternatives for bisphenol A owing to political and consumer pressure use other bisphenols to produce bisphenol A-free products.[28] Studies have shown that bisphenol F and bisphenol S also have endocrine-disrupting effects,[29,30] and studies have revealed that prenatal bisphenol S has the potential to generate hypothalamic neurogenesis.[31] Though, our study did not observe an association between bisphenol exposure during pregnancy and non-verbal IQ. More research is needed to confirm these null findings, especially because the exposure to bisphenol S and F included in this study occurred in 2004–2006; health concerns associated with bisphenol A have been growing since and have resulted in an increased use of bisphenol S and F as substitutes.[32]

Third, we found that a 10-fold higher OP pesticide exposure during late-pregnancy was associated with 4.3-point decrease in non-verbal IQ. Exposure in early and mid-pregnancy and mean exposure across pregnancy were not associated with non-verbal IQ. A similar association was observed among 7-year-old children living in the Salinas Valley of Monterey County, California (CHAMACOS cohort). A log10 increase in OP pesticide concentrations measured in late pregnancy was predictive of 3.5-point lower IQ score in this study.[33] This suggests that late pregnancy is a potential susceptible window for neurotoxic effects of OP pesticide exposure. However, more studies are needed to replicate these results.

Fourth, despite a large body of literature from both animal and epidemiological studies linking prenatal OP exposure to various behavioral outcomes, we found that OP pesticide exposure was not associated with ADHD or autistic traits in children. In this research, we

used the Child Behavior Checklist (CBCL) to measure children's ADHD symptoms at 3 years, 6 years, and 10 years of age. At the child age of 6 years, the Social Responsiveness Scale (SRS) was used to assess autistic traits. These null findings are important because of the extensive follow-up of behavior problems of children in this study, the fairly high background exposure levels of OP pesticides during gestation, and the large size of the study sample.

The observed inverse associations between phthalate and OP pesticide exposure during pregnancy and non-verbal IQ were different in different time windows of exposure. A potential mechanism that may explain the inverse association between early-pregnancy exposure to phthalates and non-verbal IQ is the disturbance of the thyroid hormone system.[34] Thyroid hormones are crucial for normal fetal brain development.[35] Notably, the earliest stages of brain development are the most susceptible to a disturbed supply of thyroid hormones.[36] During this period, the fetus is unable to produce thyroid hormones by itself and depends entirely on the maternal thyroid hormones crossing the placental barrier.[37,38] Indeed, studies have shown that prenatal exposure to phthalates— including that in early pregnancy—is related to alterations in circulating thyroid hormone levels and reduced thyroid function,[39-42] which are well-known determinants of the neurodevelopment of the offspring.[43] For instance, a previous study using data from the Generation R Study observed an association between early pregnancy maternal thyroid function and offspring neurodevelopment.[44] The investigation of the potential effects of phthalate and bisphenol exposure on thyroid function using data from the Generation R Study is underway. Future studies should investigate the potential mediating role of thyroid function in the association between prenatal phthalate exposure and offspring IQ. Regarding OP pesticides, the present study found late-pregnancy exposure to be associated with non-verbal IQ. Robust literature from animal studies suggests that OP pesticide exposure during development at fairly low-dose levels has neurotoxic effects.[45,46] The third trimester is a crucial period for normal brain development; during this timeframe, the brain increases four times in size, resulting in a significant increase in brain surface area with the formation of tertiary sulci and gyri.[47] Many biological processes such as neuronal organisation[48,49] occur during this period, and interference by chemical insults such as OP pesticides during this developmental phase may result in adverse neurodevelopmental outcomes. Further research should be carried out to confirm the observation that late-pregnancy exposure to OP pesticides is associated with cognition, especially because some other studies do not report an inverse association with IQ.[50,51]

In summary, we observed that phthalate and OP pesticide exposure during pregnancy were predictive of non-verbal IQ and that these inverse associations varied with the time window of exposure. We found no association of bisphenol exposure during pregnancy

with child non-verbal IQ. Furthermore, high gestational OP pesticide exposure was not associated with ADHD or autistic traits.

Exposure to non-persistent chemicals and brain structure, thyroid function, and fetal growth.

Because considerable uncertainty exists about which mechanisms underlie the observed associations between exposure to non-persistent chemicals and neurodevelopment, we explored whether prenatal exposure to these chemicals are associated with two important determinants of neurodevelopment and a direct assessment of neurodevelopment: thyroid function, brain structure, and fetal growth.

Thyroid function

Phthalates, bisphenols, and OP pesticides are endocrine-disrupting chemicals. Animal studies suggest that prenatal exposure to these chemicals affects thyroid function, but few studies have investigated these associations in humans.

As described above, exposure during pregnancy is particularly interesting because thyroid hormones are crucial for brain development during gestation. These chemicals have the ability to surpass the placental and blood–brain barrier and may disturb the thyroid hormone metabolism and thereby impact fetal development processes regulated by these hormones. This work investigated the association between prenatal OP pesticide exposure with first-trimester maternal and cord blood thyroid hormone concentrations (Chapter 6). In maternal and cord blood the free thyroxine (FT4) and thyroid-stimulating hormone (TSH) were determined. Further, first trimester maternal TPO antibodies (TPOAbs) and total thyroxine (TT4) were also measured. We found that prenatal OP pesticide exposure was not related to first-trimester maternal FT4, TSH, TPOAb, and TT4 concentrations. The onset of thyroid function in the human fetus occurs around 16–20 weeks of gestation.[37,52] Thus, during this early period of gestation, the fetus is dependent on the active transport of maternal thyroid hormone across the placenta. The absence of an association between prenatal OP pesticide exposure and first-trimester maternal thyroid concentrations may, therefore, indicate that first-trimester maternal thyroid function does not mediate the association between early-pregnancy OP pesticide exposure and neurodevelopment. Indeed, no association was observed between early-pregnancy OP pesticide exposure and non-verbal IQ, ADHD, and autistic traits. Similar to maternal thyroid concentrations, cord blood concentrations of TSH and FT4 showed no association with prenatal OP pesticide exposure. These results do not provide evidence for mediation by fetal thyroid function in the effect of prenatal OP pesticide exposure on offspring neurodevelopment and indicate that other mechanisms play a role in the observed association between late-pregnancy OP pesticide exposure and non-verbal IQ. As stated previously, studies investigating the potential effects of phthalate and bisphenol

exposure on thyroid function using data from the Generation R Study are ongoing. An additional study should be carried out to explore the mediating role of thyroid function in the association between prenatal phthalate exposure and offspring IQ.

Brain structure

Rauh and Margolis emphasize in their seminal research review that we lack an understanding of the brain alterations underlying the associations between prenatal exposure to environmental chemicals and neurodevelopment.[53] This review expressed the need for direct assessments of brain measurements via neuroimaging in environmental exposure studies to investigate such potential brain alterations. The Generation R Study performed magnetic resonance imaging in 9–12-year-old children to obtain T1-weighted images to estimate brain volume and surface-based cortical thickness and surface area and diffusion tensor images to measure white matter microstructure. We investigated whether prenatal exposure to phthalates (chapter 7) and OP pesticides (chapter 8) was associated with these brain measures.

Higher maternal urinary concentrations of the metabolite monoethyl phthalate was associated with lower total gray matter volume and that higher mono-isobutyl phthalate concentrations were associated with higher thalamic volume. Furthermore, higher maternal urinary concentrations of the metabolites monobenzyl phthalate and mCPP were predictive of thicker inferior parietal and mediolateral temporal cortices in the left hemisphere. There was no association between phthalate exposure and measures of white matter microstructure. Moreover, a higher mean exposure to OP pesticides across pregnancy was associated with lower white matter microstructure, and these associations were mainly driven by the early- and mid-pregnancy exposure. No associations were observed with structural brain morphology, including brain volume, cortical thickness, or cortical surface area. Notably, research to investigate the association between prenatal bisphenol exposure and brain morphology and white matter microstructure using data from the Generation R Study is currently in progress.

These findings suggest that phthalates and OP pesticides, at standard doses of exposure, contribute to altered brain development in children. However, further studies on brain imaging are warranted to test the reproducibility of these results, especially because the effect size for phthalate exposure was generally small and, as a consequence of having a broad range of metabolites and multiple brain structural outcomes, the number of tests performed were high. However, multiple testing correction may reveal whether some of these results are false positive (i.e., type 1 error). Furthermore, future work will investigate the mediating role of structural brain alterations (measured at 9 years) in the effect of pregnancy exposure to non-persistent chemicals on neuropsychological development

(measured at 13 years). This work will be performed when the IQ data of the children collected at 13 years of age are ready to use.

Fetal growth

Finally, we investigated whether exposure to non-persistent chemicals was associated with fetal growth. Altered growth during fetal development might be related to poor health in childhood and throughout the lifespan, including poor neurodevelopmental outcomes.[54,55] We found that prenatal exposure to OP pesticides was predictive of lower fetal length and weight measured during mid-pregnancy, though no association was observed at birth (chapter 9). These results were confirmed by our follow-up study in which we assessed the association between prenatal exposure to a mixture of phthalates, bisphenols, and OP pesticides and measures of fetal growth (chapter 10). The results indicated that prenatal exposure to this mixture was predictive of lower fetal size, as assessed by length and weight measures. Analyses of individual chemical classes revealed that the phthalate and OP pesticide mixtures drove the association of the overall mixture. Associations were non-linear, and the nature of the non-linearity differed when weight measurements were considered. For estimated fetal weight measured by ultrasound at 18–25 weeks, the largest difference was observed in the lower exposure range (i.e., going from only the first to the second quartile). A similar pattern was observed for weight at >25 weeks. For birth weight, however, the largest difference was observed in the higher overall exposure range (i.e., going from the third to the fourth quartile). Fetal growth changes during specific periods of pregnancy may differentially impact childhood health outcomes and may also exhibit differences in susceptibility to environmental chemical exposure. Decreased fetal development in early gestation might be critical for health consequences in childhood. For example, early pregnancy restricted fetal growth is linked to accelerated weight gain and poorer cardiovascular outcomes in children.[56,57] On the other hand, differences in birth weight were only apparent at high levels of exposure. Lower birth weight is associated with numerous health outcomes.[58,59] The observation that only high levels of exposure were associated with lower birth weight may indicate that fat mass gain from late pregnancy until birth is not as susceptible to the effect of exposure to non-persistent chemicals as weight gain during the first and second trimester. However, future studies including gestational fetal growth parameters are warranted to confirm our observations, especially because most previous studies of exposure to non-persistent chemicals and fetal growth have used growth parameters at birth only, whereas our study also included ultrasound measures in pregnancy and found effects of a chemical mixture on gestational fetal weight.

Taken together, these results demonstrate that prenatal exposure to phthalates and OP pesticides is associated with brain structural alterations and reduced fetal weight, which occur in different periods of pregnancy. As these exposures may be modifiable, these

findings have implications for preventing potential brain alterations in children and growth restriction in pregnancy. However, further research is needed to confirm our results.

Methodological considerations

The strengths and limitations of each study included in this thesis have been described in detail in the previous chapters. In this section, we will discuss the general methodological considerations regarding the measurement error inherent in the exposure biomarkers used in this work, timing of the measurement and periods of heightened susceptibility, analyses of repeated exposure measures, and the paradigm shift in risk assessment from the individual substance approach to the chemical mixture approach. These are important issues and should be considered in existing and future population studies regarding the association of prenatal exposure to non-persistent chemicals and child health outcomes.

Measurement error

To estimate the potential health impact of phthalates, bisphenols, and OP pesticides, an precise assessment of an exposure during a meaningful time window is required.[60] Most studies investigating the neurodevelopmental effects of exposure to non-persistent chemicals during pregnancy have depended on exposure concentrations measured in 1 or 2 spot urine samples. However, these chemicals are characterized by a brief half-life and are excreted in urine after only 1 or 2 days. This indicates that the biomarker concentrations might differ from day to day within each individual as a consequence of variable contact with exposure sources (e.g., variable diet patterns or use of different consumer goods) and result in high (within-subject) temporal variability[5,61-63] Thus, studies depending on one or few biospecimens may have inaccurately characterized the average exposure over a certain time period (e.g., a trimester or the whole pregnancy period) and have suffered from classical-type measurement error. Classical-type measurement error arises if the measured exposure concentrations fluctuates around the true unmeasured concentration (e.g., average exposure concentration during a trimester) in such a manner that the mean of multiple measurements is likely to estimate the correct individual exposure concentration.[64-66] Classical-type measurement error is anticipated to result into attenuation bias in dose–response associations, less precise effect estimates, and reduced statistical power.[64,67,68]

To increase accuracy, carrying out several repeated exposure measurements on each individual is a relevant possibility. However, most studies on the health effects of exposure to non-persistent chemicals have used only one or two biomarkers because collecting multiple urine specimens over time results in increased sample collection, storage, and assay costs and high participant burden. When a few biospecimens are available for each

subject, the bias in the exposure concentration can be reduced by applying a posteriori disattenuation (dividing regression coefficient by the intraclass correlation coefficient of the corresponding exposure) or use measurement error statistics.[64,69-71] Simulation extrapolation and regression calibration ar such examples.[64,69-71] Both measurement error models make use of several biospecimens per subject to correct the dose-response function for attenuation bias. Indeed, as observed in Chapter 5 and Chapter 7, correcting regression estimates by using measurement error models resulted in stronger effect estimates (i.e., further away from the null) than the estimates presented in the main model. However, the significance level was essentially the same and the standard errors were increased (i.e., more imprecise), which is a trade-off of the measurement error model application[64] and an important limitation.

One alternative method that provides the benefit of repeated exposure information without increasing analytical costs is to increase subject burden and collect more urine samples and perform within-subject biospecimen pooling.[69,72,73] In this approach, several urine samples during a relevant time window are collected and then pooled for each individual before analyzing the chemical of interest (within-subject pooling). Indeed, as theoretically validated[69] for chemicals with a known intraclass correlation (ICC) of 0.2 (which would be similar to some ICCs that we reported for the non-persistent chemicals tested in the present study), the reliance on a single urine measurement to asses exposure in a sample of 1000 participants (which approximate our study sample) will result in an attenuation bias (i.e., bias towards the null) of 79% with 13% power. By increasing the number of biospecimens to 10 per subject, the attenuation bias would be 29% with 36% power. Another approach to increase power and slightly reduce attenuation bias would be to increase sample size. If the sample size is increased to 2000 participants with five biospecimens per subject, the attenuation bias would be 44% with 54% power. Additionally, Vernet et al. (2019) carried out a validation study of the within-individual biospecimen pooling method to reduce exposure misclassification. Vernet et al. (2019) compared two innovative pooling approaches (protocol 1 and protocol 2) to estimate the chemical exposure during pregnancy with three classical approaches including the approach that was used in our work: three random spot samples were collected during pregnancy. Protocol 1 consist of collecting and within-subject pooling of complete daily spot samples during 3 weeks of pregnancy (15 ± 2, 24 ± 2, and 32 ± 1 gestational weeks). These three separate weekly pooled urine samples are then pooled again to obtain within-subject pregnancy pools for each participant. Protocol 2 followed the same approach; however, instead of collecting complete daily spot samples, three randomly selected day samples were used. They concluded that protocol 2 provided similar accurate estimate exposure averages as compared to protocol 1. When mean pregnancy exposure levels of protocol 1 were compared to those of the approach in which three random spot samples are collected during pregnancy (used in our work), they showed a relatively high correlation

(r = 0.6–0.9) for most chemicals and fair agreement. However, similar to Perrier et al. (2016), they also noted that relying on one or few biomarkers per subject for chemicals with high (within-subject) temporal variability (such as bisphenol biomarkers) results in a poor estimate of the mean exposure and leads to attenuation bias. To limit the attenuation bias below 10%, 4–6 samples across pregnancy are needed for chemicals with a relatively high ICC (0.6) such as some phthalate metabolites, whereas 18–35 samples are needed for chemicals with a relatively low ICC (0.2) such as bisphenol A.[69,72,73] Alternatively, attenuation bias could be limited by considerably increasing sample sizes in combination with the application of a posteriori disattenuation.[69]

Overall, without increased analytical biomarker expenses, a pooling approach allows to use exposure data from multiple biospecimens to estimate mean exposure values and reduce attenuation bias in dose–response associations. Recently, the within-subject pooling approach has been applied in approximately 500 pregnant women from the SEPAGES birth cohort study in France[74] and in a sample (n = 157) of the HELIX exposome project[75] in which both studies showed the possibility of executing such an approach. However, it may not always be achievable to collect a few dozen spot urine samples per participants (here pregnant women) of large population-based studies such as the Generation R Study, especially because the participant burden is already high. For example, during each visit in the Generation R Study, a broad range of biospecimens are already collected and multiple measurements are taken by several research teams. Unless it is a main priority of the principal investigators involved, additionally collecting a few dozen spot urine samples may not be achievable. Furthermore, pooling all urine samples collected during pregnancy to estimate pregnancy exposure may potentially result in diluted effects because of the existence of specific susceptible periods within pregnancy.

Timing of the measurement and periods of heightened susceptibility

The timing of the exposure measurement is essential in studies of gestational exposure to chemicals because the exposure during susceptible periods (i.e., critical windows) of fetal growth may have permanent effects throughout the life course.[76,77] Susceptible periods are developmental phases through which chemical exposure results in a larger effect on health than the exposure to the identical chemical during another period.[78] Investigating susceptible periods might be useful for studies examining chemical exposures and the health effects in children.[78] Hypothesized on prior knowledge on underlying developmental processes, researchers might compare associations of exposures measured at different time intervals to explain the potential mechanisms. Moreover, presenting associations of exposures measured at different time intervals with an outcome may help future studies to choose a potentially susceptible period. Finally, knowing the susceptible windows can provide valuable information for targeted exposure interventions in order to increase the health impact more efficiently.[78]

The existence of susceptible periods to chemical exposure may result in diluted estimations of the exposure-effect associations if studies investigating the effect of such chemicals mistime the data collection. Ideally, studies should measure the exposure during that exact time window to observe the strongest association.[79] This can be difficult because the existence, beginning, and duration of the susceptible period to a specific exposure is often unknown. Therefore, the absence of an association between exposure to a chemical and offspring health might occur because the exposure was not measured during the relevant window.[79] Furthermore, the identification of susceptible windows requires many repeated measurements of the exposure throughout development. However, most studies related to prenatal exposure to non-persistent chemicals and neurodevelopment have relied on a single biospecimen collected during pregnancy. It is likely that many of these studies may have missed the susceptible period. The present study included three repeated measures of exposure during early, mid-, and late pregnancy and found some evidence for potential susceptible periods to chemical exposure (e.g., early-pregnancy phthalate exposure). Although it is more frequent than most other studies, it is conceivable that potential windows of susceptibility were missed, and more measurements are required to observe the strongest association.

Collecting multiple biospecimens during several potential windows of susceptibility helps to identify the susceptible time window but can be logistically difficult. Frequently, for convenience, biological materials are sampled at time periods determined by logistics (e.g., routine visits at the center) rather than the expected susceptible periods. This may increase exposure misclassification and result in poor effect estimation.[79] Next, studies often investigate the health effects of prenatal chemical exposure; however, the effects of exposure to chemicals are not confined to the fetal period and may affect health throughout the developmental period. It is likely that other periods of susceptibility exist for that chemical. For example, recent studies suggest that chemical exposure disturbs oocytes or sperm, which may consequently affect offspring health via epigenetics[80,81] Moreover, susceptible periods may also be present during childhood. For example, higher lead concentrations measured in early childhood is predictive of reduced cognition[82] and bisphenol A concentrations measured during childhood was associated with behavior problems.[83]

The timing of the outcome measure in identifying susceptible periods to chemical exposure is equally important. Neurodevelopment from fetal life onwards is a complex and dynamic process. Understanding the trajectory of neurodevelopment and timing of neurodevelopmental events is essential for determining how environmental disturbances during specific developmental time windows can affect certain structures and functions. Repeated outcome measurements throughout the developmental period are needed to estimate the neurodevelopmental trajectory and onset of neurodevelopmental disorders.

Using a single outcome measurement at one point during childhood may result in an imprecise estimate of the neurodevelopmental disorder status. For example, it is conceivable that studies assessing the outcome in a single time point during early childhood may have suffered from potential outcome misclassification owing to late onset of symptoms as a consequence of neurodevelopmental disorders or a measurement problem of applying the assessment at a younger age than appropriate for a certain test (e.g., IQ test at < 4 years). Moreover, studies using a single outcome measurement may also have suffered from outcome misclassification because the developing brain may be able to adapt to chemical exposure because of its plasticity and associations between prenatal exposure and child neurodevelopment may recover as children age.[84] The present study was able to use repeated measurements for ADHD traits in children at 3, 6, and 10 years of age, which is a major strength. However, more repeated measurements from multiple neurodevelopmental outcomes would strengthen this work and help to investigate more accurately the effect of prenatal chemical exposure on neurodevelopment. Neurodevelopmental data, including brain morphology data, from the Generation R participants will be soon be available, which will allow us to perform advanced mediation analyses (e.g., to investigate whether brain morphology at 10 years is a mediator in the association of prenatal phthalate exposure and child IQ at 13 years).

Overall, this discussion highlights the importance of assessing the exposure and outcome at multiple time points during preconception (including biospecimens of the father), gestation, infancy, childhood, and adolescence. Such studies will help determine the potential windows of susceptibility to chemical exposure and help understand the trajectory of neurodevelopment and the timing of neurodevelopmental events. A continuation of studies investigating developmental processes and susceptible periods will improve future epidemiological work. Moreover, the identification of susceptible windows in epidemiological studies can inform experimental studies investigating the underlying mechanisms of increased susceptibility. However, as described above, it may not be achievable to collect a few dozen urine samples from the same women during pregnancy. Alternatively, when a certain period is suspected to be a potential window of susceptibility to chemical exposure, a design with random exposure assessments during that potential susceptible window in different women would be a possibility.

Statistical approaches to handle repeated exposure measurements

As described in the previous two sections, the few studies with multiple exposure measures over time most often used the mean value to reduce measurement error.[85,86] Although this strategy is helpful in summarizing the exposure data measured across time and reducing measurement error, studies with repeated exposure data are also interested in exploring potential windows of susceptibility during pregnancy.[78]

A frequently used method is to apply linear regression models for each time point of the exposure separately and compare the estimates of each separate model with one another. However, this method has several notable limitations. First, this approach is not able to directly test the differences in effects across time windows. Second, the existence of missing observations in exposure data at varying time points may result in different sample sizes across the regression models if not imputed. Finally, because the same outcome is used in each regression model, the hypotheses tested are not independent.[87,88] Alternatively, some studies fitted a mutually adjusted regression model in which all exposure windows are included in a single regression model. This strategy has the benefit that the independent effect of exposure for each time period can be estimated.[89,90] However, models may be reduced in sample size owing to missingness in exposure measurement at different time windows and correlated exposures may introduce multi-collinearity problems.[87,88] Furthermore, the interpretation of the effect differs with timing of exposure. For example, when early-, mid-, and late-pregnancy exposures are included in the model, the direct effect of early-pregnancy exposure not mediated through mid- and late-pregnancy exposure is estimated. For late-pregnancy exposure, only the effect not confounded by early- and mid-pregnancy exposure (equal to the direct effect) is estimated.

Several methods have been proposed as alternatives to analyze repeated exposure data and explore potential windows of susceptibility. One method regularly applied in chemical exposure studies,[25,91-93] including ours,[94] is the multiple informant method (MIM). This method was initially developed to model data from different informants or sources which are related to the same inherent construct.[95,96] However, it can also be applied in settings in which multiple measures of the exposure of the same individual are present.[87] The MIM is advantageous because it can jointly estimate separate regressions for each time period of exposure and formally test whether the effect of exposure differs across time periods. However, the MIM is not appropriate for highly correlated data, not robust to misclassification of the exposure, and has low power.[87]

Further, two-stage mixed effect models might be applied to model repeated exposure measures and a single outcome.[87,88] In this approach, the first step is to model the longitudinally time-varying exposure as a function of time (using random slopes and intercepts) to obtain subject-specific slopes and intercepts. The second step is to simultaneously fit the subject-specific slopes and intercepts as continuous predictors into the linear model with the outcome of interest.[87,88] The advantages of this method are the flexibility of modeling the participants exposure pattern across time and handling of missing exposure data at different time points. However, the interpretation of the effect estimates is not straightforward anymore.

Finally, recent novel methodological approaches have been developed to model chemical mixture exposures. Several of these approaches are able to account for repeated measurements of exposure. These methods are able to allow the effect of the exposure on the outcome to differ over time, allowing for non-linear and non-additive effects, and able to include interactions among different exposures and within exposures over time. These methods will be discussed in the next section.

From the individual substance approach to analyses of chemical mixtures

Exposure to non-persistent chemicals is universal and multisource.[97,98] Biomonitoring research have revealed that the background exposure of pregnant women and fetuses is continuous and comprises several chemicals concurrently as opposed to separate chemicals.[77,99,100] For example, in the present study, biomarker concentrations of phthalates, bisphenols, and OP pesticides were frequently detected among the same individuals. However, the majority of epidemiological studies on non-persistent chemicals have focused on the health consequences of separate chemical exposures.[101] This approach may not be ideal to the study of chemicals that can act additively, synergistically, antagonistically, or inertly in relation to a given outcome.[102,103] Furthermore, single-chemical models can bias effect estimates in the presence of co-pollutant confounders and increase false-positives when correlated exposures are modeled separately.[104] However, studies of separate pollutants who observed no associations may also have considerably underestimated the health effects of exposure.[103] For example, via a common mechanism, multiple agents can interact at smaller exposure levels to reach a similar health effect than the exposure level that is needed for every pollutant separately.[105-110]

Therefore, many researchers have stated the necessity for epidemiological research to progress from the study of adverse health outcomes of separate pollutants into the investigation of mixtures.[79,101,103,104,111,112] However, the estimation of the individual and aggregate health effects of chemical mixture exposure remains analytically challenging, and the development of statistical methods to analyze mixture effects on health and environment is an area of active and ongoing research.[101,104,113-115] Examples of statistical challenges are the presence of highly correlated chemicals from similar exposure sources, which may result in inflated standard errors and instable estimates; the possibility that one chemical can be a mediator or effect modifier in the association between another chemical and a health outcome; and the lack of power to determine small associations in the occurrence of measurement error, small sample sizes, non-linearity, and effect modification.[101,113,114]

Epidemiological data on a variety of chemicals enable researchers to address different research questions pertaining to chemical mixtures. Several statistical methods that are suited for different questions in datasets with chemical mixtures have emerged. A recent review provides detailed information regarding existing and alternative statistical methods

applied in research of gestational exposure to chemical mixtures[101], such as identifying the most potent compound within the mixture.[116,117] In this discussion, I will elaborate on two novel methods that are able to determine the joint effect of chemical exposure on health outcomes.

One innovative method for the analyses of health effects of exposure to mixtures is the Bayesian kernel machine regression (BKMR). This method is able to handle time-varying exposures to multi-pollutant mixture and allows for hierarchical individual chemical and chemical group selection.[118,119] This method simultaneously estimates the importance of chemical classes with high within group correlations in addition to the estimation of the effect of separate chemicals within a chemical class on a given outcome. BKMR is able to approximates the mixture exposure–response association by producing a single effect estimate of the overall mixture effect. The effect estimate of the overall mixture is than interpreted as the health effect that is related with all chemical concentrations at a specific quantile versus the median.[118] This method is increasingly being applied in environmental epidemiology. For example, a study from Kupsco et al. (2019) used this method to estimate the mixture effects and interaction effects of prenatal exposure to metals on childhood cardiometabolic risk.[120] Another Bayesian technique based on the kernel machine regression (KMR) framework is called lagged kernel machine regression (LKMR), which was designed to detect susceptible exposure windows of chemical mixtures on health.[78] This method is able to model non-linear effects of a mixture at any given exposure window and able to deal with interaction effects among and within exposures.[78] Notably, LKMR is most well adapted for the investigation of chemical mixtures in which the exposure is measured during few time periods.[78,121] A recent study used this method to analyze the association between exposure to four ambient pollutants and birth weight.[122] Wilson et al. (2019) aimed to simultaneously identify the windows of susceptibility to exposures and estimate the complex effects of multiple pollutants.

Another recently developed method that is able to determine the joint effect of chemical exposure on health outcomes is called the quantile g-computation method and has already been applied in several studies.[123,124] We used this method to investigate the joint effect of prenatal exposure to OP pesticides, phthalates, and bisphenols on fetal growth. Quantile g-computation approximates the difference in a certain outcome by applying a joint intervention on all chemical exposures simultaneously, while adjusting for relevant confounders.[120] Quantile g-computation specifically approximates the joint effect of increasing every chemical concentrations within the mixture by one quantile.[120] Furthermore, this method provides the opportunity to estimate the joint effect of a specific mixture (e.g., phthalate metabolites) while keeping the other chemicals (bisphenols and DAP metabolites) as a constant. Quantile g-computation is also able to deal with highly correlated exposures and does not assume directional homogeneity (i.e., the assumption

that the mutually adjusted association for every separate chemical concentration in the model with a given outcome have the same direction). Further, this method is able to account for non-linearity and non-additivity by including polynomial features, splines, and interaction terms.

Research on prenatal chemical exposure on children's health is currently moving from the study of adverse health outcomes of separate pollutants into the investigation of mixtures. Daily exposure to non-persistent chemicals such as phthalates, bisphenols and OP pesticides does not occur in isolation but as exposure to a complex mixture of chemicals that originate from similar sources. We described two novel methods that aim to determine the joint effect of chemical exposure on health outcomes. We believe that the investigation of the joint effect of chemical mixture exposure (both the whole mixture and the mixture within a chemical group) is important because it provides effect estimates that more closely correspond to real-world exposures to chemicals that initiate from similar sources; moreover, it offers easiness of inference and may directly inform potential public health interventions. For example, using such models, one can estimate the health benefits of reducing all chemical exposures or the health benefits of eliminating only low-molecular-weight phthalate exposure while other exposures (e.g., high-molecular-weight phthalates, bisphenols, and OP pesticides) are continued.

General implications

Studies included in the present thesis have important public health consequences and may help to inform public health institutions. We know that the exposure to OP pesticides, phthalates, and bisphenols is ubiquitous and occurs as exposure to mixtures. In addition, OP pesticide concentrations were 2–3 fold higher than those in most other previous studies. Furthermore, we found evidence for an association between prenatal exposure to OP pesticides and phthalates and child cognition. Depending on the timing of the exposure and the chemical, we found higher chemical exposure to be associated with a 2–4 points lower IQ score in children.

A higher IQ score during childhood is predictive of healthier behavior and lifestyle-factors later in life such as higher educational attainment, improved SES, and improved access to health services.[125,126] Multiple studies have investigated the economic consequences of exposure to chemicals by relating it to IQ loss. These studies concluded that the burden and cost of chemical exposures are high.[127] For example, in the United States each IQ point that is lost from the mean IQ of the general population is predicted to have an annual cost of US$ 71 billion.[128]

Finally, our results suggest that prenatal exposure to OP pesticides and phthalates are associated with altered fetal growth and brain development in children. These results need to be replicated by future studies, but public health institutions and clinicians should be aware of the potential neurodevelopmental effects of prenatal exposure to these chemicals. Interventions and guidelines may help to inform the general public and clinicians about the potential harmful effects of prenatal exposure to these chemicals. This could, for example, be comparable to the public health guidelines circulated by the European Food Safety Authority on fish consumption to reduce mercury exposure for pregnant women. Notably, several studies have demonstrated relatively easy interventions that are effective in lowering the exposure to these chemicals during pregnancy. For example, Lu et al. (2008) clearly demonstrated that switching to an organic diet provides a remarkable and immediate protective effect against exposure to OP pesticides, which are commonly used in agricultural production. These findings were confirmed in a recent intervention study, which found that organic diet was associated with a significant reduction in urinary excretion of several pesticide metabolites and parent compounds.[129] Dietary interventions have also been successful in lowering the exposure to phthalates and bisphenols.[19] Rudel et al. (2011) demonstrated that bisphenol and phthalate levels substantially decreased by more than half when participants changed their diet from canned and packaged foods to a fresh food diet. Furthermore, a study demonstrated that methods accessible to the general population, such as selecting cosmetics that are labelled to be without phthalates, can reduce personal exposure to these chemicals.[130]

Future directions

Based on the findings in this thesis, several directions for future research can be formulated. We found that early-pregnancy phthalate exposure and late-pregnancy OP pesticide exposure were particularly associated with IQ. It is of importance to improve these studies by increasing the number of biospecimens collected throughout pregnancy. This will help to reduce measurement error and facilitate a more detailed detection of potential windows of susceptibility. Second, we found evidence that the exposure to non-persistent chemicals is associated with altered fetal growth and brain development. These findings need to be validated by replication studies. In parallel, future studies should be carried out to investigate the potential mediating role of fetal growth and brain development in the association between chemical exposure and neurodevelopment. Third, most studies examining maternal exposure to these chemicals in the etiology of child health have been restricted to single-exposure models rather than models based on real-life mixture combinations. Currently, the field of environmental exposure and health is moving from the study of adverse health outcomes of separate pollutants into the investigation of mixtures. New techniques have emerged, which allow for modeling the effects of repeated chemical

mixture exposure on health and identifying potential periods of susceptibility. Future studies should use these techniques and investigate whether real-life prenatal chemical mixture exposure is associated with child neurodevelopment. These techniques may also assist in other research beyond the field of chemical mixtures and their health effects, for example, research on the exposome, which is defined as "the cumulative measure of environmental influences and associated biological responses throughout the lifespan, including exposures from the environment, behavior, diet, and endogenous processes."[131] Fourth, intervention studies to reduce non-persistent chemical concentrations are needed to detect potential sources of exposure and to provide health risk management strategies that may help to reduce personal or societal exposure. Finally, for epidemiological studies investigating the effect of environmental and chemical exposures on neurodevelopment, it is almost never possible to perform randomized controlled trials (RCTs) owing to ethical reasons.[132] Therefore, the research in this field depends on observational studies. Furthermore, using RCTs to prove causality of non-persistent chemicals is often problematic because these chemicals are omnipresent in humans; each person has a certain level of exposure, and the exposure itself is constant.[132] However, several recent inventive studies have used natural experiments to match RCTs.[132] For example, a study utilized the random assignment of dormitories to undergrad students at Harvard University to study the causal relationship between exposure to high temperatures during a heatwave and cognitive functioning in students.[133] Another study aiming to infer causality of dibutyl phthalate exposure on semen quality took advantage of the fact that certain mesalamine medications prescribed for inflammatory bowel disease contain phthalates in the coating though other medications do not.[134] Therefore, being exposed or not exposed (i.e., receiving versus not receiving the medication with dibutyl phthalate) relies primarily on the prescribing doctor and not on further confounders. Inventiveness in taking advantages in these random circumstances may be an extremely valuable strategy to tackle the current causal issues in epidemiological studies on the association between environmental and chemical exposures with neurodevelopment.[132]

Practical implications

To reduce personal exposure to OP pesticides, phthalates, and bisphenols, the following general suggestions should be considered. First, using organic products in one's general diet (or replacing some food products with organic products) has been shown to substantially reduce the urine levels of OP pesticides. Furthermore, reducing the exposure to phthalates can be achieved by replacing packaged food products by fresh foods and using phthalate-free personal care products. Exposure can be further reduced by choosing shower and window curtains made from PVC-free alternatives such as linen, cotton, or bamboo instead of from PVC-containing products, and selecting flooring made from natural materials

instead of vinyl flooring. Finally, a broad range of potentially phthalate-containing general products used by young children—such as teething toys and teethers, soft plastic bath toys, and baby shampoo—could easily be replaced by safe products made from wood, 100% natural rubber, or cotton. Regarding bisphenols, diet is the main route of exposure, and the level of exposure can be considerably reduced by avoiding canned food and beverages. Furthermore, bisphenols are present in thermal paper used for a variety of products such as ATM receipts, supermarket receipts, boarding passes, and movie tickets. A recent study tested 170 thermal paper cash register receipts from 62 different retailers such as supermarkets, fast food diners, gas stations, and banks for the presence of bisphenols.[135] They found that almost all receipts (n = 168) had detectable bisphenol concentrations. An easy approach to avoid such exposure to bisphenols is declining the receipt from the cashier; when possible, it is better to ask for a digital receipt, purchase digital movie tickets, and use an online boarding pass.

Conclusion

In summary, the findings presented in this thesis suggest that prenatal exposure to non-persistent chemicals is negatively associated with child neurodevelopment. Politicians, industries, and public health institutions should not ignore the potential risk of prenatal exposure to chemicals such as OP pesticides and phthalates, which are likely associated with fetal growth, brain development, and IQ.

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12

Chapter 12

Summary / Samenvatting

Summary

During pregnancy, women are universally exposed to a broad range of chemicals such as phthalates, bisphenols, and organophosphate (OP) pesticides, commonly present in consumer products. Animal studies have linked low-dose exposure to these chemicals during pregnancy to adverse effects on the neurodevelopment of the offspring. In some cases, the few epidemiological studies investigating fetal exposure to these chemicals are suggestive of an effect on neurodevelopment but are overall inconclusive. Further, considerable uncertainty exists about which mechanisms underlie the observed associations between exposure to non-persistent chemicals and neurodevelopment. The present thesis examined the relationship between prenatal exposure to the nonpersistent chemicals phthalates, bisphenols, and OP pesticides and neurodevelopment in children by:

(i) studying the determinants of exposure to non-persistent chemicals during pregnancy
(ii) exploring the association of prenatal exposure to non-persistent chemicals with neurodevelopment in children
(iii) investigating the effect of exposure to these non-persistent chemicals on the potential mediators—such as thyroid function, brain structure, and fetal growth—of the association with neurodevelopment.

These aims were explored using data from the Generation R Study, which is characterized by a large sample size; detailed follow-up information of prenatal and postnatal development of the fetus and child, respectively; repeated measurements (early, mid-, and late-pregnancy) of exposure to non-persistent chemicals; and availability of detailed demographic information. The results of this thesis are summarized below.

In **Part II** we explored the determinants of exposure to non-persistent chemicals during pregnancy. **Chapter 2** presents the potential determinants of prenatal exposure to OP pesticides. In this chapter OP pesticide concentrations were frequently detected in urine samples collected during early-, mid-, and late-pregnancy and these concentrations were 2–3 times higher than those in most other studies previously conducted. Adverse maternal lifestyle- and socioeconomic status (SES)-related factors were associated with lower OP pesticide exposure concentrations and fruit intake was the main dietary source of exposure to OP pesticides. This chapter provides useful insights into the potential determinants of exposure to OP pesticides and strengthens the hypothesis that dietary intake is predictive of OP pesticide exposure among pregnant women living in an urban environment. The potential determinants of phthalate and bisphenol exposure has been examined by an earlier study using data from the Generation R Study cohort. These results are discussed in **Chapter 11**.

Part III examines the association between prenatal exposure to non-persistent chemicals and neurodevelopment in children. **Chapter 3** explores the association between prenatal exposure to phthalates and bisphenols and offspring non-verbal intelligence quotient (IQ). Phthalate exposure during early pregnancy was consistently associated with lower non-verbal IQ score in children. Fewer inverse associations were observed for exposure in late pregnancy and mean exposure across pregnancy. Bisphenol exposure during pregnancy was not associated with non-verbal IQ. These results are important because all other previous studies assessing the association between prenatal phthalate exposure and IQ have examined the exposure during mid- or late-pregnancy and, therefore, may explain why these studies have reported diverse findings. These results therefore suggest that early pregnancy is a susceptible period. **Chapter 4** expands on this study by investigating the association between OP pesticide exposure during pregnancy and offspring non-verbal IQ. In this study we found that higher late pregnancy OP pesticide exposure was associated with lower non-verbal IQ score in children. Early and mid-pregnancy OP pesticide exposure was not associated with non-verbal IQ. These results suggest that late pregnancy is a potential susceptible window for neurotoxic effects of OP pesticide exposure. Beside cognition, non-persistent chemical exposure may be associated with neurobehavioral problems. **Chapter 5** explored whether OP pesticide exposure is associated with attention-deficit hyperactivity disorder (ADHD) and autistic traits in children. Despite a large body of literature from both animal and epidemiological studies linking prenatal OP exposure to various behavioral outcomes, no association between OP pesticide exposure and ADHD or autistic traits was observed. These null findings are important because of the extensive follow-up measurements of behavior problems of children in this study, the relatively high background exposure levels during gestation, and large sample size.

Part IV investigates the effect of exposure to non-persistent chemicals on the potential mediators—such as thyroid function, brain structure, and fetal growth—of the association with neurodevelopment. **Chapter 6** presents the results of prenatal OP pesticide exposure and first-trimester maternal or cord blood thyroid hormone concentrations. We found no association between prenatal OP pesticide exposure and first-trimester maternal thyroid function or fetal thyroid function. These results may indicate that first-trimester maternal thyroid function does not mediate the association between early pregnancy OP pesticide exposure and neurodevelopment. Further, these results do not support mediation by fetal thyroid function in the effect of prenatal OP pesticide exposure on child neurodevelopment. Other mechanisms may therefore play a role in the observed association between late pregnancy OP pesticide exposure and non-verbal IQ. In **Chapter 7** we investigated another potential mediator and explored whether prenatal exposure to phthalates was associated with brain morphology and white matter microstructure in children. Maternal urinary concentrations of monoethyl phthalate metabolite concentrations were associated with

lower total gray matter volume and monobutyl phthalate with larger thalamus volumes. There was no association between phthalate exposure and measures of white matter microstructure. These findings suggest that phthalates exposure contributes to altered brain development in children. **Chapter 8** expands on this by investigating the association between OP pesticide exposure during pregnancy and offspring brain morphology and white matter microstructure. Higher mean exposure to OP pesticides across pregnancy was associated with lower white matter microstructure, and these associations were mainly driven by the early and mid-pregnancy exposure. No associations were observed between prenatal OP pesticide exposure and structural brain morphology, including brain volume, cortical thickness, or cortical surface area. This study provides the first evidence that OP pesticides may alter normal white matter microstructure in children, which could have consequences for normal neurodevelopment. Besides thyroid function and brain morphology, fetal growth may also be a potential mediator in the association between prenatal exposure to non-persistent chemicals and neurodevelopment. **Chapter 9** therefore investigated whether exposure to non-persistent chemicals was associated with fetal growth. We found that prenatal exposure to OP pesticides was predictive of lower fetal weight and length measured during mid-pregnancy, but not at delivery. These results were confirmed by our follow-up study presented in **Chapter 10** in which we assessed the association between prenatal exposure to a mixture of phthalates, bisphenols, and OP pesticides and measures of fetal growth. The results indicated that prenatal exposure to this mixture was predictive of lower fetal size, as indicated by length and weight measurements. Analyses of individual chemical classes revealed that the phthalate and OP pesticide mixtures drove the association of the overall mixture.

Finally, in **Chapter 11** we first discussed the main findings of this thesis. We then discussed the general methodological considerations, the implications of this study, and provide future directions and recommendations. We finalize this chapter by providing general suggestions to the reader to reduce personal exposure to OP pesticides, phthalates, and bisphenols.

Samenvatting

Tijdens de zwangerschap worden vrouwen universeel blootgesteld aan een breed scala van chemicaliën zoals ftalaten, bisfenolen en organofosfaat (OP) pesticiden. Deze chemische stoffen komen voornamelijk voor in consumentenproducten. Dierstudies hebben een verband aangetoond tussen de prenatale blootstelling van een lage dosis aan deze chemicaliën en nadelige effecten op de neurologische ontwikkeling van het nageslacht. In sommige gevallen suggereren de enkele epidemiologische studies dat de blootstelling van de foetus aan deze chemicaliën een effect heeft op de neurologische ontwikkeling. Echter, zijn deze epidemiologische studies over het algemeen niet doorslaggevend. Verder bestaat er onzekerheid over welke mechanismen ten grondslag liggen aan de waargenomen associaties tussen de blootstelling aan niet-persistente chemicaliën en de neurologische ontwikkeling. Dit proefschrift onderzocht de relatie tussen prenatale blootstelling aan de niet-persistente chemicaliën ftalaten, bisfenolen en OP pesticiden en de neurologische ontwikkeling van kinderen door:

i) het onderzoeken van mogelijke determinanten van blootstelling aan niet-persistente chemicaliën tijdens de zwangerschap
(ii) het onderzoeken van de associatie tussen prenatale blootstelling aan niet-persistente chemicaliën en neurologische ontwikkeling van kinderen
(iii) het onderzoeken van het effect van blootstelling aan deze niet-persistente chemicaliën op potentiële mediatoren—zoals schildklierfunctie, hersenstructuur en foetale groei—van de associatie met neurologische ontwikkeling.

Deze doelen werden onderzocht met behulp van gegevens uit de Generation R Studie, die wordt gekenmerkt door een grote steekproefomvang; gedetailleerde follow-up informatie van de prenatale en postnatale ontwikkeling van respectievelijk de foetus en het kind; herhaalde metingen (vroege, midden- en late zwangerschap) van blootstelling aan niet-persistente chemicaliën; en beschikbaarheid van gedetailleerde demografische informatie. De resultaten van dit proefschrift worden hieronder samengevat.

In **deel II** werden de determinanten van blootstelling aan niet-persistente chemicaliën tijdens de zwangerschap onderzocht. In **Hoofdstuk 2** onderzochten wij de mogelijke determinanten van prenatale blootstelling aan OP pesticiden. In dit hoofdstuk werden OP pesticiden concentraties veelvuldig aangetroffen in urinemonsters verzameld tijdens de vroege, midden en late zwangerschap. Deze concentraties waren 2-3 keer hoger dan de concentraties gevonden in de meeste eerder uitgevoerde onderzoeken. Ongunstige levensstijl- en sociaaleconomisch status-gerelateerde factoren waren geassocieerd met lagere concentraties van OP-pesticiden. Daarnaast was de consumptie van fruit tijdens

de zwangerschap de belangrijkste voedselbron van blootstelling aan OP-pesticiden. Dit hoofdstuk biedt inzichten in de potentiële determinanten van blootstelling aan OP pesticiden en versterkt de hypothese dat de consumptie van voedsel voorspellend is voor blootstelling aan OP-pesticiden bij zwangere vrouwen die in een stedelijke omgeving wonen. De mogelijke determinanten van ftalaten en bisfenolen blootstelling zijn onderzocht door een eerdere studie met behulp van gegevens van het Generation R Study-cohort. Deze resultaten worden besproken in **hoofdstuk 11.**

Deel III onderzocht de associatie tussen prenatale blootstelling aan niet-persistente chemicaliën en neurologische ontwikkeling bij kinderen**.** In **Hoofdstuk 3** keken wij naar de associatie tussen prenatale blootstelling aan ftalaten en bisfenolen en het non-verbale intelligentiequotiënt (IQ) van het nageslacht. Blootstelling aan ftalaten tijdens de vroege zwangerschap was consistent geassocieerd met een lagere non-verbale IQ-score bij kinderen. Er werden minder negatieve associaties waargenomen voor blootstelling tijdens de late zwangerschap en voor gemiddelde blootstelling gedurende de zwangerschap. Blootstelling aan bisfenolen tijdens de zwangerschap was niet geassocieerd met non-verbale IQ. Deze resultaten zijn belangrijk omdat alle eerdere studies die de associatie tussen prenatale ftalaten blootstelling en IQ onderzochten, de blootstelling tijdens het midden of laat in de zwangerschap hebben gemeten. Dit verklaart mogelijk waarom deze onderzoeken geen consistente resultaten hebben gevonden. Deze resultaten suggereren daarom dat vroege zwangerschap een gevoelige periode is voor de neurotoxische effecten van blootstelling aan ftalaten. **Hoofdstuk 4** is een uitbreiding op deze studie waarin we het verband onderzochten tussen blootstelling aan OP pesticiden tijdens de zwangerschap en het non-verbale IQ van het kind. In deze studie vonden wij dat een hogere blootstelling aan OP-pesticiden in de late zwangerschap geassocieerd was met een lagere non-verbale IQ-score bij kinderen. Blootstelling aan OP-pesticiden in het begin en midden van de zwangerschap was niet geassocieerd met non-verbale IQ. Deze resultaten suggereren dat de late zwangerschap een potentieel gevoelig periode is voor de neurotoxische effecten van blootstelling aan OP-pesticiden. Naast cognitie keken wij ook naar het verband met neurologische gedragsproblemen. In **Hoofdstuk 5** bestudeerden wij of blootstelling aan OP pesticiden geassocieerd is met symptomen van ADHD en autisme bij kinderen. Ondanks dat zowel dierstudies als eerdere epidemiologische studies associaties vonden, werd er in onze studie geen verband waargenomen tussen blootstelling aan OP-pesticiden en symptomen van ADHD of autisme. Deze nulresultaten zijn belangrijk vanwege de uitgebreide follow-upmetingen van gedragsproblemen van kinderen, de relatief hoge achtergrondblootstellingsniveaus tijdens de zwangerschap en de grote steekproefomvang.

Deel IV presenteert het onderzochte effect van blootstelling aan niet-persistente chemicaliën op potentiële mediatoren van de associatie met neurologische ontwikkeling. In **Hoofdstuk 6** onderzochte wij de associatie tussen prenatale blootstelling aan OP pesticiden en

schildklierhormoon concentraties. Er werd geen verband gevonden tussen blootstelling aan OP-pesticiden tijdens de zwangerschap en de schildklierhormoon concentraties van de moeder gemeten in het eerste trimester en de schildklierhormoon concentraties gemeten in het navelstrengbloed. Deze resultaten suggereren dat de schildklierfunctie van de moeder in het eerste trimester en foetale schildklierfunctie niet het verband medieert tussen blootstelling aan OP-pesticiden tijdens zwangerschap en neurologische ontwikkeling. Andere mechanismen spelen daarom mogelijk een rol in de associatie tussen blootstelling aan OP-pesticiden tijdens de late zwangerschap en non-verbale IQ. In **Hoofdstuk 7** werd er gekeken naar een andere mediator en onderzocht of prenatale blootstelling aan ftalaten geassocieerd was met de hersenstructuur en de witte stof microstructuur. Maternale mono-ethyl ftalaat metaboliet concentraties waren geassocieerd met een kleiner volume van de totale grijze stof, en monobutyl ftalaat metaboliet concentraties waren geassocieerd met een groter volume van de thalamus. Er was geen verband tussen blootstelling aan ftalaten en metingen van de witte stof microstructuur. Deze bevindingen suggereren dat blootstelling aan ftalaten hersenontwikkeling bij kinderen beïnvloed. **Hoofdstuk 8** is een uitbreiding op deze studie waarin we de associatie onderzochten tussen blootstelling aan OP pesticiden tijdens de zwangerschap en de hersenstructuur en de witte stof microstructuur van kinderen. Een hogere gemiddelde blootstelling aan OP-pesticiden tijdens de zwangerschap was geassocieerd met een lagere microstructuur van witte stof, en deze associaties werden voornamelijk veroorzaakt door blootstelling aan het begin en midden van de zwangerschap. Er werden geen associaties waargenomen tussen prenatale blootstelling aan OP-pesticiden en structurele breinstructuur, inclusief hersenvolume, corticale dikte en corticale oppervlakte. Deze studie levert het eerste bewijs dat blootstelling aan OP-pesticiden de normale microstructuur van witte stof bij kinderen kunnen beïnvloeden, wat gevolgen kan hebben voor de normale neurologische ontwikkeling. Naast de schildklierfunctie en de morfologie van de hersenen, kan de groei van de foetus ook een potentiële mediator zijn in het verband tussen prenatale blootstelling aan niet-persistente chemicaliën en neurologische ontwikkeling. **Hoofdstuk 9** onderzochten wij daarom of de blootstelling aan niet-persistente chemicaliën geassocieerd was met foetale groei. We ontdekten dat prenatale blootstelling aan OP-pesticiden voorspellend was voor een lager foetaal gewicht en lengte gemeten halverwege de zwangerschap, maar niet bij de bevalling. Deze resultaten werden bevestigd door onze vervolgstudie gepresenteerd in **Hoofdstuk 10** waarin we de associatie tussen prenatale blootstelling aan een mix van ftalaten, bisfenolen en OP pesticiden en foetale groei hebben onderzocht. De resultaten gaven aan dat prenatale blootstelling aan deze mix voorspellend was voor een kleinere foetale grootte. Ten slotte hebben wij in **Hoofdstuk 11** de belangrijkste bevindingen van dit proefschrift besproken. Vervolgens hebben we de algemene methodologische overwegingen en de implicaties van deze studie besproken en toekomstige aanwijzingen en aanbevelingen gepresenteerd. We ronden dit hoofdstuk af met algemene suggesties

aan de lezer om de persoonlijke blootstelling aan OP-pesticiden, ftalaten en bisfenolen te verminderen.

Appendix

Acknowledgements
Author affiliations
Publications not part of this thesis
Portfolio
Words of gratitude / Dankwoord
About the author

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List of publications not part of this thesis

Koopman-Verhoeff, M. E., **van den Dries, M. A.**, van Seters, J. J., Luijk, M., Tiemeier, H., & Luik, A. I. (2019). Association of Sleep Problems and Melatonin Use in School-aged Children. *JAMA pediatrics*, 173(9), 883–885.

de Lijster, J. M., **van den Dries, M. A.**, van der Ende, J., Utens, E., Jaddoe, V. W., Dieleman, G. C., Hillegers, M., Tiemeier, H., & Legerstee, J. S. (2019). Developmental Trajectories of Anxiety and Depression Symptoms from Early to Middle Childhood: a Population-Based Cohort Study in the Netherlands. *Journal of abnormal child psychology*, 47(11), 1785–1798.

Rodriguez-Ayllon, M., Derks, I., **van den Dries, M. A.**, Esteban-Cornejo, I., Labrecque, J. A., Yang-Huang, J., Raat, H., Vernooij, M. W., White, T., Ortega, F. B., Tiemeier, H., & Muetzel, R. L. (2020). Associations of physical activity and screen time with white matter microstructure in children from the general population. *NeuroImage*, 205, 116258.

PhD Portfolio

Name PhD student:	Michiel Arjen van den Dries
Erasmus MC Department:	Child & Adolescent Psychiatry/Psychology
PhD period:	Feb 2016 – Jun 2020
Promotor:	Prof. dr. H. Tiemeier,
Copromotors:	Dr. M. Guxens
	Dr. ir. A. Pronk

1. PhD training	Year	ECTS
Courses		
Advanced Causal Inference	2017	1.3
Causal Mediation Analysis, Erasmus MC	2018	1.4
Advances in Clinical Epidemiology, Erasmus MC	2018	0.7
Erasmus Summer Lectures, Erasmus MC	2018	0.4
Masterclass: Advances in Genomics Research, Erasmus MC	2018	0.4
Child Psychiatric Epidemiology, Erasmus MC	2019	0.9
Skills courses		
Radiation protection expertise level 5R	2016	1.0
Research Integrity, Erasmus MC	2018	0.3
International conferences		
ISES, Utrecht, the Netherlands *(poster presentation)*	2016	1.0
EDCEH, Rennes, France	2018	1.0
ISEE, Utrecht, the Netherlands *(oral & poster presentation)*	2019	1.4
Symposia, meetings & workshops		
Generation R Behavioral Group Meetings, Rotterdam, the Netherlands *(oral presentation)*	2016-2020	1.0
Generation R Research Meetings, Rotterdam, the Netherlands *(oral presentation)*	2016-2020	1.0
ACTION meeting, Amsterdam, the Netherlands	2017	0.3
EEARN meeting, Rotterdam, the Netherlands	2018	0.3
NYU Langone Health, Department of Pediatrics, New York, USA *(oral presentation)*	2019	1.0
NIEHS reproductive Lunch Meetings	2019	0.3
NIEHS mixtures Working Group Meetings *(oral presentation)*	2019	1.0
NIEHS Summer Internship Program Poster Session	2019	0.3
11th Annual NIEHS Epidemiology Branch Science day, Durham, USA. (*presentation judge*)	2019	0.3
ATHLETE kick-off meeting, Barcelona, Spain	2020	0.6

2. Teaching activities		
Courses		
Biostatistical methods I, SPSS/R *(research assistance)*	2016	1.7
Clinical trials and diagnostic tests *(research assistance)*	2017	0.6
Biostatistical methods I, SPSS/R *(research assistance)*	2017	0.6
Supervision Master thesis		
Judith van Seters (Medical Sciences, Erasmus Medical Center). *Association of sleep problems and melatonin use in school-aged children*	2017-2018	3.0
Duifje M.B. Wurzer, (Clinical Psychology, Erasmus University Rotterdam). *Prenatal Organophosphate Pesticide Exposure and Academic Achievement in Preadolescence: A Population-based Cohort Study in the Netherlands*	2018-2019	3.0
Gelitza Croes (Clinical Psychology, Erasmus University Rotterdam). *The Association of Prenatal Organophosphate Pesticides Exposure with Neuromotor Development in Young Children*	2018-2019	3.0
3. Other activities		
Generation R general tasks *(360 dayparts of data-collection)*	2016-2019	
Peer review *Neurotoxicology (two reviews)*	2016-2017	1.2
Peer review *Environment International (three reviews)*	2018	1.8
Peer review *Environmental Epidemiology (two reviews)*	2019-2020	1.2
Three months research visit at the National Institute of Environmental Health Sciences (NIEHS)	2019	
4. Grants and prices		
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1 ECTS (European Credit Transfer System) is equal to a workload of 28 hours

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– Johan Cruijff

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– Theo Maassen

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About the author

Michiel Arjen van den Dries was born in Bilthoven, the Netherlands. After his bachelor program in Health Sciences at the VU University of Amsterdam, Michiel started two masters: a two-year research master in Epidemiology with the specialization Occupational and Environmental Epidemiology at the University of Utrecht, and a two-year research master in Management, Policy-Analysis and Entrepreneurship in Health and Life Sciences with the specialization in International Public Health at the VU University of Amsterdam. During his masters, the interests in scientific research developed and in 2016 he started a PhD program supervised by Prof. dr. Henning Tiemeier, Dr. Mònica Guxens, and Dr. ir. Anjoeka Pronk at the Department of Child and Adolescent Psychiatry, and the Generation R Study Group at the Erasmus MC in Rotterdam, from which the results are presented in this thesis. During his PhD he was invited as a visiting scientist at the National Institute of Environmental Health Sciences (NIEHS), Durham, NC, United States, to perform a research project. In 2020 Michiel van den Dries is working at ISGlobal, Barcelona, Spain, as a postdoctoral researcher. He is working on a project about prenatal exposure to non-persistent chemical mixtures and neurodevelopment in children which is funded by two research grants he received: The LifeCycle project fellowship, and The Academy Ter Meulen grant. Michiel will pursue his scientific career as a postdoctoral researcher in the field of environmental exposures and children's health at ISGlobal and hopes to eventually start his own research group.