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

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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 pesticides exposure, particularly in vulnerable populations, the primary sources of exposure remain unclear. The present study was designed to investigate the levels of DAP metabolites concentrations across pregnancy and to examine various determinants of DAP 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



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**Competing interests**

The authors did not declare any competing interests.

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 the 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 less toxic metabolites remains unclear. Further research will be undertaken to investigate the possible effects of this relatively high level OP pesticides exposure on offspring health.

### Keywords

Environmental exposure Organophosphate pesticides Dialkyl phosphate metabolites Pregnancy Biomonitoring Cohort study

## 1. Introduction

In the Netherlands, more than 50% of the total surface area is used for agriculture purposes (LNV, 2010). Organophosphate (OP) pesticides are a class of insecticides that are commonly used in agriculture and, between 1998 and 2008, approximately 35% of the insecticides used in the Netherlands were OP pesticides (CBS, 2017), which may lead to high background exposure.

For non-occupationally exposed individuals, the exposure occurs most likely through the ingestion of food (Lu et al., 2008). Further, residential exposure can occur through use of insecticides in and around the house (Julien et al., 2007; Lu et al., 2004; Valcke et al., 2006; Whyatt et al., 2003). Exposures to high doses of OP pesticides are known to be neurotoxic in humans and animals (Costa, 2006; Pope et al., 1992; Rosenstock et al., 1991). Nevertheless, results obtained from both animal and human studies raise concerns about the potential health impact of low-level OP pesticides exposure in the general population (Jaga and Dharmani, 2003).

Animal studies have demonstrated that OP pesticide exposure levels even below the threshold for acetylcholinesterase inhibition can alter psychological disorder related gene expression (Savy et al., 2018), induce changes in behavior and neurochemistry (Savy et al., 2015), and result in cognitive impairments (dos Santos et al., 2016; Terry, 2012). Moreover, low level OP pesticide exposure can change neuronal cell development (Slotkin et al., 2008), induce oxidative stress (Slotkin and Seidler, 2010; Zafiropoulos et al., 2014), and influence the thyroid hormone levels and the reproductive system (Androutsopoulos et al., 2013; De Angelis et al., 2009; Haviland et al., 2010).

Fetuses and children are more susceptible to neurotoxic effects than adults as the human brain is particularly vulnerable during maturational and developmental processes (Rice and Barone, 2000). 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 (Bradman et al., 2003). Further, epidemiological studies suggest that prenatal exposure to OP pesticides may be associated with adverse neurodevelopmental and birth outcomes (González-Alzaga et al., 2014; Harley et al., 2016), although results are not conclusive (Engel et al., 2016).

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 (Barr et al., 2006; Duggan et al., 2003). These DAP metabolites have a short half-life and are mostly excreted in urine within 24 h (Huen et al., 2012). 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 (Margariti et al., 2007).

Several studies investigating prenatal OP pesticides 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 (Lewis et al., 2015; Llop et al., 2017; Sokoloff et al., 2016; Yolton et al., 2013). 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 (Spaan et al., 2015; Ye et al., 2008).

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 pesticides exposure in pregnant women is (Llop et al., 2017). 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 (Lewis et al., 2015; Sokoloff et al., 2016; Yolton et al., 2013). Moreover, several studies, including our pilot study, investigated only broad food group categories (e.g., fruit) (Spaan et al., 2015; Yolton et al., 2013) while few studies explored specific food items (e.g., apples) (Llop et

al., 2017; Sokoloff et al., 2016). The sample size of most studies limited the ability to test specific determinants of DAP metabolite concentrations (Lewis et al., 2015; Spaan et al., 2015; Yolton et al., 2013). 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.

## 2. Methods

### 2.1. Study population and follow-up

The Generation R Study is a prospective population-based birth cohort designed to identify the early environmental and genetic determinants of normal and abnormal development and health from fetal life onwards (Kooijman et al., 2016). 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 these, 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 pesticides 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 women with 1 sample).

### 2.2. Urine collection and analysis of DAP metabolites

Details of maternal urine specimen collection have been described elsewhere (Kruithof et al., 2014). 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) (Health Canada, 2010).

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 provides information about the total exposure to OP pesticides that generate DAP metabolites (Margariti et al., 2007).

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 (Butler, 1975), 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 (Spaan et al., 2015; Ye et al., 2008), 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) (Koo and Li, 2016). 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 (Mukaka, 2012).

### 2.3. Determinants of OP pesticides 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) (Steenweg-de Graaff et al., 2012). 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 (Slimani et al., 2002). Average daily energy intake was calculated using the Dutch food composition Table 2006. More details about the assessment of dietary intake are described elsewhere (Steenweg-de Graaff et al., 2012). All food items were adjusted for energy intake (varying between 619 and 3452 kcal). Except for the household income (13%), owning a dog (11%), owning a cat (11%), occupational exposure to pesticides (14%), partner's exposure to pesticides (31%), and the maternal dietary determinants (22%), the percentage of missing values of these variables did not exceed 10%.

### 2.4. 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 (Palarea-Albaladejo and Martín-Fernández, 2015). 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 statistical 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/√2 is another common substitution method in environmental exposure studies (Baccarelli et al., 2005), we substituted values below LOD with LOD/√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 (O'Brien et al., 2015). 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) (R core Team, 2015).

## 3. Results

### 3.1. 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.

### 3.2. DAP metabolite levels in urine

Table 2 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 compared with 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 (Spaan et al., 2015). 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) (Koo and Li,

2016). Moreover, in accordance with the Pearson correlation coefficients, both the total DAP metabolite concentrations in nmol/L (r = 0.14–0.24) and in nmol/g creatinine (r = 0.17–0.34) across pregnancy, showed weak correlations (Mukaka, 2012).

### 3.3. Predictors of urinary OP pesticides metabolite levels

**3.3.1. 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 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 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.

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.

**3.3.2. 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.

### 3.4. Sensitivity analysis

The results were consistent when the LOD/√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 Tables 4 and 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 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

Tables 4 and 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).

## 4. 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 compared to other American and European studies (Fig. 1). 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, Quebec) as this study (Sokoloff et al., 2016). 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 (Eskenazi et al., 2007), NHANES study: median = 72 nmol/g creatinine, 52 nmol/L (Ye et al., 2009), Mount Sinai cohort: 82 nmol/L (Engel et al., 2007), and HOME cohort: median = 81 nmol/L (Rauch et al., 2012)), European studies (France PELAGIE cohort: median = 44 nmol/L (Cartier et al., 2015), Norwegian MoBa cohort: GM = 145 nmol/g creatinine, 87 nmol/L (Ye et al., 2009), and Spanish INMA cohort: GM = 107 nmol/g creatinine, 96 nmol/L (Llop et al., 2017)) and compared to a study from Thailand (median = 161 nmol/g creatinine, 90 nmol/L (Kongtip et al., 2014)). In contrast, the DAP metabolite concentrations from our study were considerably lower than those observed in China (median = 296 nmol/L (Liu et al., 2016)).

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 their 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 (Agudo, 2005; Kimmons et al., 2009).

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 (Dekker et al., 2015), an important source of OP pesticides exposure (Lu et al., 2008). 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 (Conklin et al., 2014; Heo et al., 2011) and dietary patterns are strongly related to SES, ethnic differences, and BMI (Brenner et al., 2011; Dekker et al., 2015; Martikainen et al., 2003; Newby et al., 2003). 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 (OECD, 2015). 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%) (CBS, 2017). 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 (Llop et al., 2017).

The DAP metabolite concentrations measured in our study however, were considerably lower compared to the levels observed in China and Taiwan (Huang et al., 2017; Liu et al., 2016; Zhang et al., 2014). This may be explained by China’s heavy use of OP pesticides in agricultural activities (Wu et al., 2010). 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 (Wang et al., 2013).

Our results are in agreement with findings obtained by Bradman et al. (2003), Llop et al. (2017), Lu et al. (2008), Sokolofff 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 pesticides 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 that 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 pesticides residue levels (ChemKap, 2017; European Commission, 2006). 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 (ChemKap, 2017). 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., Di-methoate, 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) (ChemKap, 2017). 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 (ChemKap, 2017).

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 (Lu et al., 2004). 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 (Lee et al., 2010). 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 (Lee et al., 2010). The observed DAP metabolite concentrations across BMI categories could also be explained by diet and SES although we adjusted for these variables. But it may also be that women with higher BMI will

excrete more creatinine (Sinkeler et al., 2011), 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 (Lu et al., 2005; Quirós-Alcalá et al., 2012). The extent to which DAP metabolite concentrations reflect exposure to the active parent pesticide rather than to less toxic metabolites remains therefore unclear (Krieger et al., 2012). Nevertheless, the measurement of DAP concentrations is scientifically accepted as a useful tool to identify and compare degrees of OP pesticides exposure in diverse populations (Bravo et al., 2004).

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 all OP pesticides (Margariti et al., 2007) 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 (Berman et al., 2016; Curl et al., 2015; Oates et al., 2014). 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 (Barr et al., 2005; Boeniger et al., 1993; Cornelis et al., 1996). Although, time of sample collection is unlikely to confound the association between possible determinants of OP pesticides 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-today variability in exposure within subjects (Needham, 2005). 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 (González-Alzaga et al., 2014; Llop et al., 2017; Sokoloff et al., 2016) 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% (Maikranz et al., 1989), 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 pesticides exposure and were able to account for various confounding variables.

We should, however, be cautious when generalizing the factors associated with increased OP pesticides 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 pesticides exposure could be different.

Overall, this study strengthens the hypothesis that dietary intake plays an important role in prenatal OP pesticides 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 (Engel et al., 2016; González-Alzaga et al., 2014; Harley et al., 2016). Considerably higher DAP metabolite concentrations were found in the Generation R population compared to these earlier cohorts. Further research will be undertaken to

investigate the possible health effects of this relatively high level of OP pesticides exposure in the offspring of the Generation R study, an urban population-based cohort.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ijheh.2018.01.013.

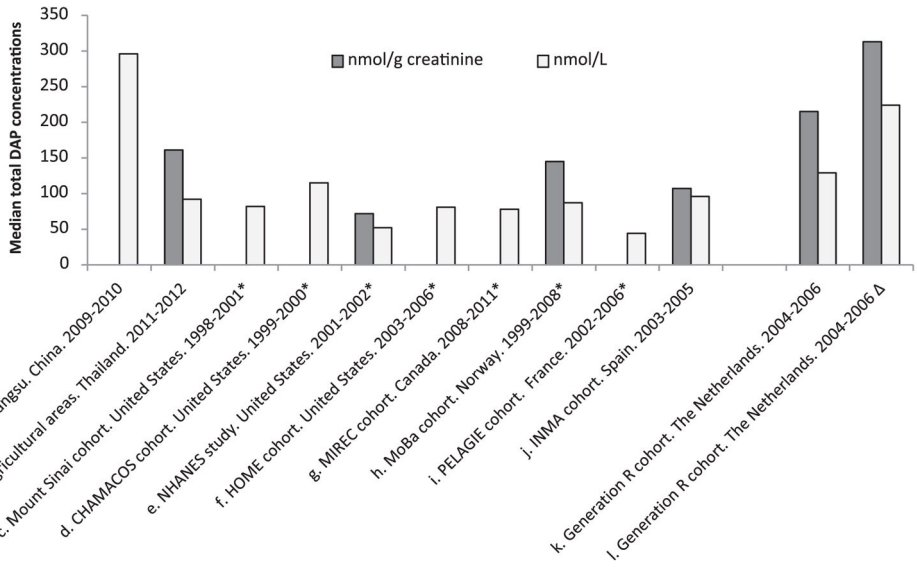


**Fig. 1.**

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 (Liu et al., 2016). 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. (Kongtip et al., 2014). 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 (Engel et al., 2007). 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 (Eskenazi et al., 2007). 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 (Ye et al., 2009). 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 (Rauch et al., 2012). g. Maternal-Infant Research on Environmental Chemicals study. One spot urine sample collected during the first trimester from 1884 women (Sokoloff et al., 2016). h. A pregnancy cohort in Norway. Ten pools of one 1-ml urine samples from 11 women at 17 weeks of gestation (Ye et al., 2009). i. Mother child cohort in Brittany, France. One spot urine sample collected at < 19 weeks of gestation from 231 women (Cartier et al., 2015). 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) (Llop et al., 2017). 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 represents the average of the three median DAP metabolite concentrations (Spaan et al., 2015; Ye et al., 2008). 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 represents the average of the three median 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 [a] (N = 9778) | Included in the study [a] (N = 784) | DAP exposure [b] 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 | |

| Characteristic | Descriptive statistics | | |
|---|---|---|---|
| | Generation R cohort [a] (N = 9778) | Included in the study [a] (N = 784) | DAP exposure [b] Median (P25, P75) (N = 784) |
| 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 | |
| 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 beverage 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 | |

| Characteristic | Descriptive statistics | | |
|---|---|---|---|
| | Generation R cohort [a] (N = 9778) | Included in the study [a] (N = 784) | DAP exposure [b] Median (P25, P75) (N = 784) |
| 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] Values shown are percentages.

[b] 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).

## Table 2

Descriptive statistics of DAP metabolite concentrations in nmol/g creatinine from 784 pregnant women from the Netherlands participating in the Generation R cohort.

| Metabolite | %<LOD | GM | min | P50 | max | 0 1 10 100 1.000 10.000 |
|---|---|---|---|---|---|---|
| **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 | 0% | 129.78 | 3.83 | 129.77 | 1547.11 | |
| 18–25 weeks | 0% | 136.05 | 12.31 | 136.54 | 1528.82 | |
| >25 weeks | 0% | 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 | |

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-Fernández, 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.

**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.

| Determinants | Total dialkyl phosphates [a] | | Dimethyl alkyl phosphates [b] | | Diethyl alkyl phosphates[c] | |
|---|---|---|---|---|---|---|
| | 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 | – | |

| Determinants | Total dialkyl phosphates [a] | | Dimethyl alkyl phosphates [b] | | Diethyl alkyl phosphates[c] | |
|---|---|---|---|---|---|---|
| | **B (95%CI)** | **P** | **B (95%CI)** | **P** | **B (95%CI)** | **P** |
| *Dutch* | **ref** | | **ref** | | **ref** | |
| 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 between the intake of food groups per 100 g/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.

| Food intake [a] | Total dialkyl phosphates [b] | | Dimethyl alkyl phosphates [c] | | Diethyl alkyl phosphates [d] | |
|---|---|---|---|---|---|---|
| | B (95%CI) | P | B (95%CI) | P | B (95%CI) | P |
| **Per 100 g/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.

* $p < 0.05$.

**Table 5**

Associations between the intake of fruit types per 100 and dialkyl phosphates metabolite concentrations on a creatinine basis (nmol/g creatinine) across pregnancy among 610 pregnant women participating in the Generation R cohort.

| Fruit intake [a] | Total dialkyl phosphates [b] | | Dimethyl alkyl phosphates [c] | | Diethyl alkyl phosphates [d] | |
|---|---|---|---|---|---|---|
| | B (95%CI) | P | B (95%CI) | P | B (95%CI) | P |
| Mandarin, per 100 g/d | 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, per 100 g/d | 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, 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 100 g/d | 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, yes | 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, per 100 g/d | 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, yes | 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, yes | 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, yes | −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, yes | 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, 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) | 0.153 | 0.024 (−0.014 to 0.062) | 0.207 | 0.037 (−0.008 to 0.083) | 0.107 |
| Strawberry/raspberry, yes | 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, yes | 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, yes | 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, yes | 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.