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

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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.

1. 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 (Hertz-Picciotto et al., 2018a). Some populations are occupationally exposed, but the exposure of pregnant women most likely occurs through their diet (Llop et al., 2017; Sokoloff et al., 2016; Lewis et al., 2015; van den Dries et al., 2018). Importantly, OP pesticides can cross the placental and blood-brain barriers and may interfere with optimal fetal development at different levels (Bradman et al., 2003).

Fetal growth and differentiation of almost all tissues, including the

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brain, adipose tissue and bone is regulated by thyroid hormones (Yen, 2001). 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 (Thorpe-Beeston et al., 1991). 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 (Glinoer, 1999). 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 (Bernal, 2005; Lavado-Autric et al., 2003). 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 (Slotkin and Seidler, 2007; Verma et al., 2009). Both prenatal thyroid hormone shortage and exposure to OP pesticides have been associated with adverse neurobehavioral and birth outcomes in children (Korevaar et al., 2018a; Munoz-Quezada et al., 2013; Hertz-Picciotto et al., 2018b; Harley et al., 2015; Todd et al., 2019). Therefore, it has been hypothesized that disruption of thyroid function is a potential mechanism relating prenatal OP pesticides exposure to brain development (Ghassabian and Trasande, 2018).

Results from animal studies suggest that organophosphates might interfere with thyroid function, although findings are inconclusive. Some studies in adult animals report an increased (Maiti and Kar, 1997) or decreased (Yadav and Singh, 1987) serum total thyroxine (TT4) concentration after exposure to dimethoate or malathion, respectively, whereas chlorpyrifos-methyl, malathion, or monocrotophos did not affect TT4 concentrations (Jeong et al., 2006; Sinha et al., 1992; Zhang et al., 2013; Zhang et al., 2014). 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 (Zhang et al., 2014), but did not differ after exposure to dimethoate or chlorpyrifos-methyl (Maiti and Kar, 1997; Jeong et al., 2006).

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 (Campos and Freire, 2016). 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 (Needham, 2005). 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 (Wang et al., 2017), all other studies but one have been performed in adult males and reported contradictive results (Campos and Freire, 2016). Higher urinary DAP metabolite concentrations were associated with higher TT4 and TSH in occupationally exposed males (Lacasaña et al., 2010). 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 (Meeker et al., 2006). 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 (Fortenberry et al., 2012). 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 (Eskenazi et al., 2007; Ye et al., 2009; Cartier et al., 2015; Liu et al., 2016). We hypothesized that higher OP pesticide exposure would be associated with lower FT4 and higher TSH concentrations.

2. Methods

2.1. Participants

This study was embedded in Generation R, a prospective population-based cohort from fetal life onwards (Kooijman et al., 2016). 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 9778 mothers who participated in the study, 8879 (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 4918 women were enrolled. Of these, 2083 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 = 1449), 800 were randomly selected for measurements of urinary dialkylphosphate (DAP) metabolites (van den Dries et al., 2018). Of these, 784 had a sufficient urine volume for analyses and 730 women also 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.

2.2. 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) (Health, 2010). 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) (van den Dries et al., 2018). To account for urinary dilution, OP pesticide metabolite concentrations were divided by urinary creatinine concentrations, which were determined based on the Jaffe reaction (Butler, 1975). The ranges

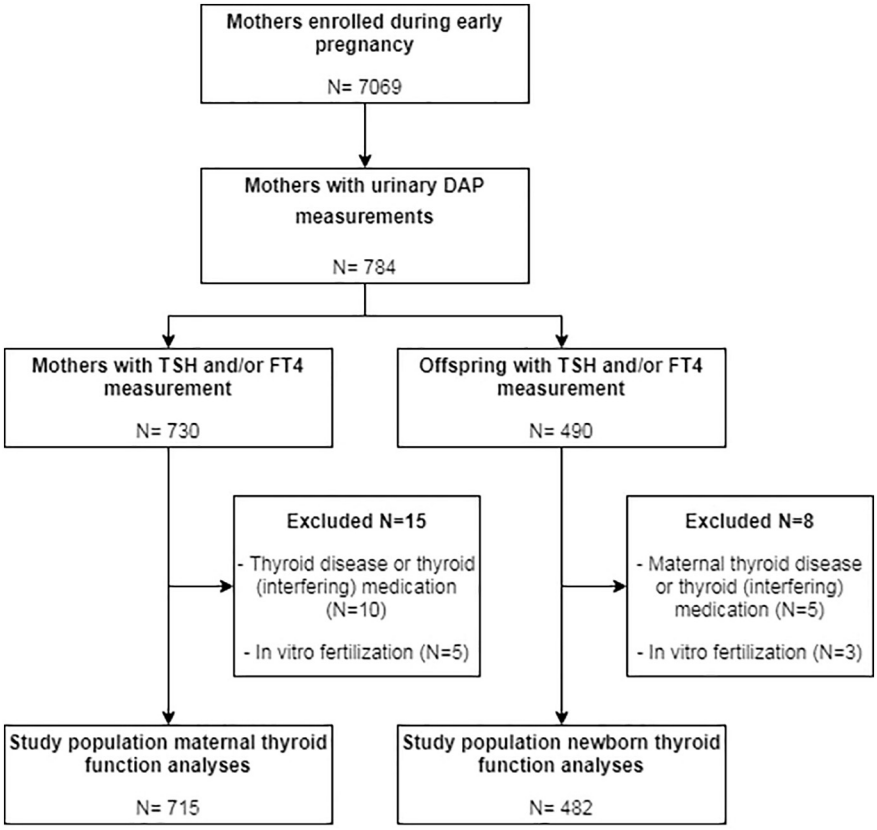

Fig. 1. Flowchart of the study population.

(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 (van den Dries et al., 2018; Kruithof et al., 2014).

2.3. 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 (Medici et al., 2012).

2.4. Covariates

Potential confounders were selected a priori based on previous studies of OP pesticides and thyroid function (Campos and Freire, 2016; Wang et al., 2017). 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 (Statistics Netherlands, 2004). 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 (Medici et al., 2014; Levie et al., 2018).

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 (Verburg et al., 2008). Except for smoking (8%) and fruit intake (22%), missing data on covariates were all < 1%.

2.5. 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

Table 1
Descriptive characteristics of mother and child pairs.

Characteristic	Maternal thyroid hormones available (n = 715)		Cord blood thyroid hormones available (n = 482)	
	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	295	61.2
1	190	26.6	131	27.2
≥2	83	11.6	56	11.6
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
Energy adjusted fruit intake, g/d	157.3	(11.5–437.8)	151.1	(11.6–437.8)
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	(79.0–1343.5)	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	(2.68–36.67)
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	(76.4–568.6)	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.

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 (Wang et al., 2017; Lacasaña et al., 2010). 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

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

Dialkyl phosphates *First urine collection phase, < 18 weeks gestation*	Mean TSH		Mean FT4		Mean TT4	*p*
	β (95% CI)	*p*	β (95% CI)	*p*	β (95% CI)	
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 (−4.04 to 5.51)	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.

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 (Korevaar et al., 2018b). 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 (Pearce, 2012; Taylor et al., 2016). 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 (Team RC, 2015; Sv and Groothuis-Oudshoorn, n.d.). 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 (van den Dries et al., 2018). 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 $P < 0.05$.

3. 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 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).

4. Discussion

In the current study, gestational OP pesticide exposure, as assessed

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

	Mean TSH		Mean FT4	
Dialkyl phosphates	β (95% CI)	*p*	β (95% CI)	*p*
Average concentrations during pregnancy				
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.

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 (Sokoloff et al., 2016; Needham, 2005). 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 (van den Dries et al., 2018). 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 (Eskenazi et al., 2007; Ye et al., 2009; Cartier et al., 2015; Liu et al., 2016). 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 (van den Dries et al., 2018).

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 (Wang et al., 2017). 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 (ChemKap, 2017; *PAN International Consolidated List of Banned Pesticides: Pesticide Action Network International*, 2017). 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 (Yu et al., 2016). 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 (Lacasaña et al., 2010) and TSH (Lacasaña et al., 2010; Meeker et al., 2006), and negative associations with FT4 (Meeker et al., 2006; Fortenberry et al., 2012) and TSH (Fortenberry et al., 2012). 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 (Jeong et al., 2006; De Angelis et al., 2009). 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 (Goldner et al., 2010). Prior studies assessed OP pesticides exposure only once and only one study focused on OP pesticide exposure during pregnancy (Wang et al., 2017; Lacasaña et al., 2010; Meeker et al., 2006; Fortenberry et al., 2012). 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 (Bajoria and Fisk, 1998), 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 (Vulsma et al., 1989). Although transfer of FT4 from mother to fetus decreases throughout pregnancy alongside an increased production of fetal thyroid hormones (Thorpe-Beeston et al., 1991), 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 (De Angelis et al., 2009; Slotkin et al., 2013). 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 (Maiti and Kar, 1997) or decreased (Yadav and Singh, 1987) serum total thyroxine (TT4) concentration after exposure to dimethoate or malathion, respectively, whereas chlorpyrifos-methyl, malathion, or monocrotophos did not affect TT4 (Jeong et al., 2006; Sinha et al., 1992; Zhang et al., 2013; Zhang et al., 2014). 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 (Zhang et al., 2014), but no differences in serum TSH concentrations were observed after exposure to dimethoate or chlorpyrifos-methyl (Maiti and Kar, 1997; Jeong et al., 2006). 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 (Margariti et al., 2007). 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) (van den Dries et al., 2018). 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 (ChemKap, 2017). 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 1 mg/kg and 2 mg/kg, respectively (Maiti and Kar, 1997; De Angelis et al., 2009; Slotkin et al., 2013). The acceptable daily intake (ADI) concentration for those organophosphates is 0.01 and 0.002 mg/kg in humans (EU pesticides database [Internet], 2016). 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 (Ishihara et al., 2003). 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 (Zhang et al., 2013).

In our study population, OP pesticides exposure during pregnancy most likely occurs through diet, with fruit intake being the main source of exposure (van den Dries et al., 2018). 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 (Zimmermann and Kohrle, 2002). 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 (Mughal et al., 2018). Our study was performed in an iodine-sufficient area, which may mitigate the effects of OP pesticides on thyroid function (Ghassabian et al., 2014). 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 (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 unclear (Krieger et al., 2012). Yet, the estimation of urinary DAP metabolite concentrations is considered a non-invasive and useful biomarker for OP pesticides exposure (Bravo et al., 2004) and is therefore the most-used method of estimating exposure to this class of compounds in general populations (Engel et al., 2016). 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 (Barr et al., 2005; Boeniger et al., 1993; Cornelis et al., 1996). 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 (Ehrenkranz et al., 2015).

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 (Lee et al., 2009). 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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Declaration of competing interest

None.

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Appendix A. Supplementary data

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