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

Michiel A. van den Dries[a,b], Sander Lamballais[b,c,d], Hanan El Marroun[a,e,f], Anjoeka Pronk[g], Suzanne Spaan[g], Kelly K. Ferguson[h], Matthew P. Longnecker[i], Henning Tiemeier[a,j], Mònica Guxens[a,k,l,m]

[a]Erasmus MC, University Medical Center Rotterdam, Department of Child and Adolescent Psychiatry, Rotterdam, the Netherlands, 3015 CN. [b]Erasmus MC, University Medical Center Rotterdam, The Generation R Study Group, Rotterdam, the Netherlands, 3015 CN. [c]Erasmus MC, University Medical Center Rotterdam, Department of Epidemiology, Rotterdam, the Netherlands, 3015 CN. [d]Erasmus MC, University Medical Center Rotterdam, Department of Clinical Genetics, Rotterdam, the Netherlands, 3015 CN. [e]Erasmus MC, University Medical Center Rotterdam, Department of Pediatrics, Rotterdam, the Netherlands, 3015 CN. [f]Department of Psychology, Education and Child Studies, Erasmus School of Social and Behavioral Sciences, Erasmus University Rotterdam, the Netherlands, 3062 PA. [g]Department of Risk Analysis for Products in Development, TNO, Zeist, the Netherlands, 3704 HE. [h]Epidemiology Branch, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, Durham, North Carolina, USA, NC 27709. [i]Ramboll, Raleigh, North Carolina, USA, NC 27612. [j]Department of Social and Behavioral Science, Harvard TH Chan School of Public Health, Boston, USA, MA 02115. [k]ISGlobal, Barcelona, Spain, 08003. [l]Pompeu Fabra University, Barcelona, Spain, 08002. [m]Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Instituto de Salud Carlos III, Spain, 28029.

Corresponding author:

Mònica Guxens

Barcelona Institute for Global Health – Campus Mar

Doctor Aiguader, 88, 08003 Barcelona.

Tel. +34 932 147 330 | Fax +34 932 147 302 | Email: monica.guxens@isglobal.org

Conflict of interest:

The authors declare no conflict of interest.

Abstract

Background

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

Method

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

Results

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

Conclusions

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

Keywords: Organophosphate pesticides; prenatal exposure; MRI; brain structural alterations; neurodevelopment

Funding: This research received financial support from the intramural research program of the National Institute of Environmental Health Sciences, National Institutes of Health (Grant# HHSN273201500003C). The general design of the Generation R Study is made possible by financial support from the Erasmus Medical Center, Rotterdam; the Erasmus University Rotterdam; Netherlands Organization for Health Research and Development (ZonMw); the Netherlands Organization for Scientific Research (NWO); and the Ministry of Health, Welfare and Sport. Neuroimaging was supported by the ZonMw TOP project no. 91211021, and super computing computations for imaging processing were supported by the NWO Physical Sciences Division (Exacte Wetenschappen) and SURFsara (Cartesius compute cluster, https://www.surf.nl). Henning Tiemeier was supported by a grant of the Netherlands Organization for Scientific Research (NWO grant No. 024.001.003, Consortium on Individual Development, and NWO/ZonMW grant 016.VICI.170.200). Mònica Guxens is funded by a Miguel Servet fellowship (MS13/00054, CPII18/00018) awarded by the Spanish Institute of Health Carlos III. Monica Guxens acknowledges support from the Spanish Ministry of Science and Innovation and State Research Agency through the "Centro de Excelencia Severo Ochoa 2019-2023" Program (CEX2018-000806-S), and support from the Generalitat de Catalunya through the CERCA Programme. Dr. Hanan El Marroun was supported by Stichting Volksbond Rotterdam, the Dutch Brain Foundation (De Hersenstichting, project number GH2016.2.01) and the European Union's Horizon 2020 research and innovation programme (grant agreement No. 733206 LifeCycle). Funders for this research had no participation in the design and conduct of the study.

Ethics approval and consent to participate: Human subjects review for the procedure of this study was carried out and approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam (IRB Registration no.: IRB00001482, MEC-2012-165, MEC-2007-413,

MEC, 217.595/2002/202, and MEC 198.782.2001.31). Written informed consent for the children and mothers was provided by the mothers.

1. Introduction

Organophosphate (OP) pesticides are chemical agents often used in agriculture to protect crops against insects. At present, five billion pounds of pesticides are being applied worldwide and approximately 33% are OP pesticides (Mahajan et al. 2019). Similarly, between 1998 and 2008 one third of the insecticides used in the Netherlands were OP pesticides (CBS 2020). In the past decade, the use of OP pesticides has been declining in both the Netherlands and European Union (EU) due to stricter legislations. However, several OP pesticides such as malathion are currently approved by the EU and OP pesticide residues are frequently detected on tested vegetables and fruits coming from importation (ChemKap 2017; EU Pesticides database 2020).

Since OP pesticide residues may persist on or in food after crop harvesting (Eaton et al. 2008), there is an increasing concern about their potential harmful health effects. The exposure to OP pesticides generally occurs through the consumption of food (Lu et al. 2008). However, residential exposure can also occur through the use of insecticides in and around the house or by living in close proximity to agricultural lands were OP pesticides are being applied (Fenske et al. 2002; Julien et al. 2007; Lu et al. 2000; Lu et al. 2004; Valcke et al. 2006; Whyatt et al. 2003).

It is well established that the exposure to high concentrations of OP pesticides is neurotoxic to both humans and animals (Costa et al. 2008; Eaton et al. 2008). However, evidence exist that OP pesticide exposure at fairly low-dose levels may also have a negative health effect (Savy et al. 2018; Slotkin et al. 2008). OP pesticides are able to pass the placental and the blood-brain barrier (Bradman et al. 2003) and, during gestation, the development of the human brain is especially susceptible to neurotoxic effects (Rice and Barone 2000). Therefore, pregnancy exposure to low-dose levels of OP pesticides might affect fetal normal brain development.

Although several epidemiological studies have reported associations between pregnancy OP pesticide exposure and offspring's neuropsychological development (Sapbamrer and Hongsibsong 2019), much uncertainty exists about the brain structural alterations underlying these associations. Magnetic resonance imaging (MRI) is a useful instrument for addressing these knowledge gaps and can help identify the associations between neurotoxic exposures and brain development (Rauh and Margolis 2016). In humans, altered brain morphology and white matter microstructure is associated with impaired cognition, behavior problems, and neurodevelopmental disorders (Dennis and Thompson 2013; Gilmore et al. 2018; Lebel and Deoni 2018; Mizuno et al. 2019). So far, only few animal studies and one small epidemiological study have investigated the relation of OP pesticide exposure on morphological brain measures. Experimental animal studies showed that OP pesticide exposure was associated with smaller brain volume, both thinning and thickening of the cortex, and alterations of white matter microstructure (Mullins et al. 2015; Roy et al. 2004; Roy et al. 2005). In humans, prenatal exposure to the OP pesticide chlorpyrifos measured in cord blood was associated with thinner cortices and alterations in cortical surface area in 40 children at 6-11 years old (Rauh et al. 2012). However, this previous human study only analyzed a specific OP pesticide, was restricted to a small sample size, and was unable to investigate the exposure across the entire pregnancy. Moreover, no previous epidemiological study investigated the association between prenatal OP pesticide exposure and white matter microstructure, which has been observed in a previous animal study (Mullins et al. 2015).

Therefore, the objective of this study was to determine whether maternal OP pesticide metabolite concentrations in urine repeatedly measured during gestation are associated with brain morphology and white matter microstructure in 518 preadolescents aged 9-12 years.

Understanding the association between prenatal OP pesticide exposure and brain morphology and white matter microstructure may help explain the association between pregnancy OP pesticide exposure and offspring's neuropsychological development observed in previous studies. Further, findings of the present study may assist in future policies regarding the regulation of OP pesticide application.

2. Materials and Methods

2.1. Study population and follow-up

This research was embedded in the Generation R Study, a population-based cohort from early fetal life onwards in Rotterdam, the Netherlands, which has been described in detail previously (Kooijman et al. 2016). Figure S1 presents a flowchart of this study. Briefly, all pregnant women who lived in the study area in Rotterdam, the Netherlands and were expected to have a delivery between 2002 and 2006 were eligible. A total of 8879 women were enrolled during pregnancy. A random sample of 800 mother-child pairs were selected for assessment of OP pesticide metabolites among the 1449 that provided three spot urine samples during pregnancy and had child's neurodevelopmental data at postnatal visits. Of those, 518 children were included in the present study as they had good quality data on MRI measurements at 9-12 years old. Human subjects review for the procedure of this study was carried out and approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam (IRB Registration no.: IRB00001482, MEC-2012-165, MEC-2007-413, MEC, 217.595/2002/202, and MEC 198.782.2001.31). Written informed consent for the children and mothers was provided by the mothers.

2.2. Urine collection and analysis of OP pesticide metabolites

A more detailed description of urine specimen collection and measurement of OP pesticide metabolites have been published previously (van den Dries et al. 2018) and can be found in the supplement (Methods S1). Briefly, 6 non-specific urinary dialkylphosphate (DAP) metabolites of OP pesticides were measured from urine samples collected at <18, 18-25, and >25 weeks of gestation by gas chromatography coupled with tandem mass spectrometry (GC–MS/MS). These include 3 diethyl alkyl phosphate (DE) and 3 dimethyl alkyl phosphate (DM) metabolites. Creatinine concentrations were also measured in order to correct for urinary dilution. All urine samples had detectable concentrations of most metabolites. The intraclass correlation for DAP metabolite concentrations was weak for a single concentration (0.22-0.26) and moderate for the average of the 3 concentrations (0.51-0.54)(van den Dries et al. 2019).

2.3. Magnetic Resonance Imaging

Details of the neuroimaging acquisition and processing can be found in the supplemental material (Methods 2). The global brain metrics derived from T_1-weighted images included total brain volume, cerebral and cerebellar white and grey matter volume, and subcortical grey matter volume. Additionally, we focused on the corpus callosum and the subcortical regions: amygdala, caudate nucleus, hippocampus, pallidum, putamen, nucleus accumbens and the thalamus (Muetzel et al. 2019). Surface-based thickness and surface area maps were made of the cerebral cortex (Muetzel et al. 2019). Diffusion tensor imaging (DTI) images were used to fit diffusion tensors at each voxel and fractional anisotropy (FA) and mean diffusivity (MD) were computed (Cook et al. 2006). Twelve major white matter tracts were identified via probalistic tractography with the FSL plugin AutoPtx (de Groot et al. 2015; Muetzel et al. 2017). These included the forceps minor and major, and the bilateral tracts of the cingulum bundle, corticospinal tract, the inferior/superior longitudinal fasciculi and the uncinate fasciculus. The mean FA and MD per tract, weighted by connectivity distribution, were then

computed. A confirmatory factor analysis was performed to model a single latent FA and MD measures across the 12 tracts, which represented global FA and MD across the brain (Muetzel et al. 2015). Global FA indicates the tendency for preferential water diffusion in white matter tracts. A lower FA score indicates in general that the comprising axons are less densely packed and the directionality of the water diffusion is not uniformly directed as compared with well-organized tracts. Global MD describes the magnitude of average water diffusion in all directions within brain tissue, with higher values generally occurring in white matter tracts that show a less well-organized structure (Alexander et al. 2007).

2.4. Potential confounders

Potential adjustment variables were selected a priori defined as the minimal sufficient adjustment set with a Directed Acyclic Graph (DAG) using the Dagitty software (Textor et al. 2017). The DAG was based on previous studies of prenatal OP pesticide exposure and neurodevelopment and on biologically plausible covariate–exposure and covariate–outcome associations observed in our data (see Figure S2). We further included adjustment variables that are ancestor of the exposure and ancestor of the outcome to increase precision. The adjustment variables were household income (less than 1200 euro/month (i.e., less than the social security level of the Netherlands), 1200 – 2000 euro/month, more than 2000 euro/month), highest achieved level of education (low (less than 3 years of high school), intermediate (3 or more years of secondary education), and high (university degree or higher vocational training)), ethnical background (Dutch, other Western, and non-Western), maternal age at enrolment, marital status (married/ living with partner versus single), parity (0, 1, or 2 or more children), smoking habits during pregnancy (none, only until pregnancy known, or continued after pregnancy known), and gestational alcohol use (none, only until pregnancy known, continued infrequently (<1 glass/week) or continued regularly ((≥1 glass/week), pre-pregnancy body

mass index (BMI) (kg/m^2), maternal IQ (assessed when the mother-child pairs visited the research center for the 6-year examination and measured by using the computerized Ravens Advanced Progressive Matrices Test, set I (Prieler 2003)), child sex, and the child age at the MRI scan.

2.5. Statistical methods

Total DE (nmol/l) was defined as the sum of diethylphosphate, diethylthiophosphate, and diethyldithiophosphate metabolite concentrations. Total DM (nmol/l) was created by summing dimethylphosphate, dimethylthiophosphate, and dimethyldithiophosphate metabolite concentrations. Total DAP (nmol/l) was created by summing the 6 metabolites. These concentrations were creatinine adjusted (nmol/g creatinine) and transformed using a log transformation (base 10) to improve linearity of the dose-response relation and model fit. Few concentrations were missing because of an inadequate sample or machine error. We therefore imputed missing concentrations (<1%) and missing confounder information 10 times by using the Multivariate Imputation by Chained Equations (MICE) package in R (R core Team 2015; van Buuren and Groothuis-Oudshoorn 2011). We included total brain volume and global FA in the imputation procedure as predictors, but we did not impute brain measures.

As a first step, we applied linear regression to examine the association of averaged DE, DM, and DAP concentrations over pregnancy with brain volumes. To account for multiple testing (14 tests for each exposure), we applied the false discovery rate correction. As a second step, we investigated metabolite concentrations – brain volume associations for each exposure time point (early, mid, and late pregnancy) separately. We performed this second step for the identification of possible periods of susceptibility and to have the ability to compare our findings with studies that only used one urine sample during gestation to measure the exposure

to OP pesticides. These analyses were also corrected for multiple testing using the FDR correction. The same multiple linear regressions were applied for the DAP – global white matter tract (FA and MD) associations. Post-hoc analyses were run on the 12 major individual white matter tracts when the analysis yielded a significant association between prenatal DAP metabolite concentrations and global white matter tracts. We explored whole-brain vertex-wise statistics using the QDECR R package (https://qdecr.com) for total DE, DM, and DAP metabolite concentrations in association with local cortical thickness and cortical surface area. Vertex-wise analyses were corrected for multiple testing by the application of Gaussian Monte Carlo null-Z simulations (The cluster-forming threshold defined as $p < 0001$). Next. these analyses were also corrected by applying a Bonferroni adjustment for the analyses of both hemispheres.

All models were adjusted for potential confounders described above. Additionally, we adjusted models of subcortical and cerebellar volumes for intracranial volume to ascertain relativity to head size. Models of the other volumes were not adjusted for intracranial volume as they were highly correlated (between $r = 0.81$ and $r = 0.93$).

As sensitivity analyses, first, we investigated potential effect modification by sex via interaction terms (P-value for interaction<0.05) to compare our results with previous studies who observed sex specific effects (Rauh et al. 2012; Sagiv et al. 2019). Second, we applied inverse probability weighting to adjust all models for loss to follow-up and to deal with potential selection bias because participants included in this study were older, had higher educational level, and more frequently Dutch as compared to the complete Generation R Study cohort (van den Dries et al. 2018). Third, because diet and the intake of healthy nutrients may confound the association between prenatal OP pesticide exposure (e.g., residues on fruits) (van den Dries et al. 2018) and

brain development (e.g., healthy nutrients) (Figure S2), we performed a sensitivity analyses in which we additionally adjusted for maternal fruit and vegetables intake. The consumption of fruit and vegetables was assessed in the first trimester using a modified version of a validated food frequency questionnaire and was adjusted for energy intake (Steenweg-de Graaff et al. 2012).

3. Results

3.1. Descriptive analysis

The median age of the mothers at enrolment was 31.2 years (IQR=5.4) and the median age of the child at MRI assessment was 9.8 years (IQR=0.3) (Table 1). The majority of mothers participating in this study were ethnically Dutch (61.4%), were nulliparous (66.4%), were none smokers (79.1%), had a high educational level (60.2%), and had a high income (73.6%). Total DAP metabolite concentrations comprised mostly DM metabolite concentrations (Table 2). The median nmol/g creatinine concentrations were comparable across the three sampling periods. The median total brain volume was 1209 cm^3 (IQR=140 cm^3) and median FA was 0.0 (IQR=2.3) (Table S1).

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

		Median (25^{th}, 75^{th} percentile) or %
Maternal characteristics		
Age		31.2 (28.6, 34.0)
	Missing	-
Ethnicity		
	Dutch	61.4%
	Other western	13.1%
	Non-western	25.5%
	Missing, N	-
Educational level		
	Low	11.2%
	Intermediate	28.6%
	High	60.2%
	Missing, N	11
Income		
	<1,200	11.6%
	1,200-2,00	14.8%
	>2,000	73.6%
	Missing, N	52
Non-verbal IQ		100.0 (90.0, 107.0)
	Missing, N	7
Body mass index		23.0 (21.2, 25.9)
	Missing, N	2
Parity		
	0	66.3%
	1	24.2%
	>=2	9.5%
	Missing, N	2
Smoking use during pregnancy		
	No smoking during pregnancy	79.1%
	Until pregnancy recognized	8.8%
	Continued during pregnancy	12.1%
	missing	40
Alcohol consumption during pregnancy		
	No consumption during pregnancy	33.2%
	Until pregnancy recognized	17.7%
	Continued occasionally	42.1%
	Continued frequently	7.0%
	Missing, N	21
Child characteristics		
Child age at assessment		9.8 (9.6, 9.9)
	Missing, N	-
Child sex		
	Male	49.4%
	Female	50.6%
	Missing, N	-

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

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

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

3.2. OP pesticide metabolite concentrations and brain volume

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

Table 3. Adjusted* association between averaged log10 transformed maternal concentrations of DM† and DE‡ metabolite concentrations in nmol/g creatinine and brain volumes (N=441) and white matter microstructure (N=474) assessed at child age 10 years.

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

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

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

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

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

§ Additionally adjusted for intracranial volume

3.3. OP pesticide metabolite concentrations and white matter microstructure

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

Regarding the individual 12 major white matter tracts, we observed that DM metabolite concentrations averaged across pregnancy were associated with lower FA and higher MD in most of the tracts except for the uncinate fasciculus tract of left hemisphere, the forceps major, and the corticospinal tract of the right hemisphere (Figure 1 and Table S4). We observed that higher averaged DE metabolite concentrations were associated with lower FA in the superior longitudinal fasciculus tract and the corticospinal tracts, and with higher MD in the cingulate gyrus of the cingulum tract of the left hemisphere, the forceps minor tract, and the inferior longitudinal fasciculus tract of the left hemisphere (Figure 1 and Table S5). The results of the total DAP metabolite concentrations were also almost identical to the results observed for the DM and DE metabolite concentrations together (Table S6).

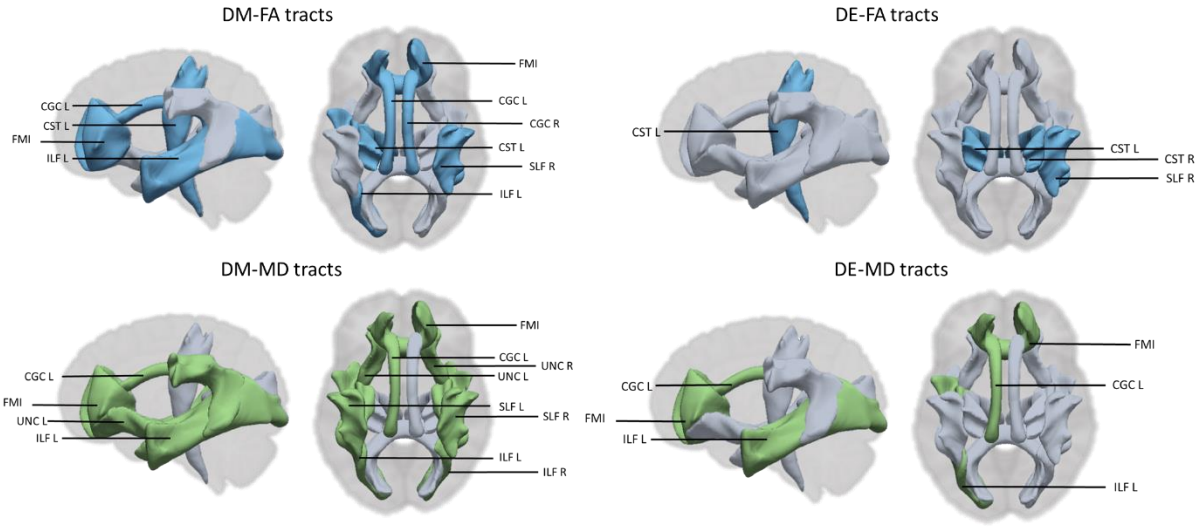

Figure 1. The white matter tracts of global fractional anisotropy (FA) and global mean diffusivity (MD) that are associated (blue= negative, green = positive) with maternal dimethyl (DM) and diethyl (DE) alkyl phosphate metabolite concentrations in nmol/g creatinine. CGC= Cingulum bundle, CST= corticospinal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

3.4. OP pesticide metabolite concentrations and cortical surface area and cortical thickness

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

3.5. Sensitivity analyses

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

4. Discussion

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

Although prior studies have noted the importance of the use of neuroimaging tools to address existing research gaps by identifying structural neurotoxic effects of prenatal OP pesticide exposure on the brain (Rauh and Margolis 2016), only one small epidemiological study has investigated this research question (Rauh et al. 2012). This study observed that prenatal exposure to chlorpyrifos, which devolves into the DE metabolites diethylphosphate and diethylthiophosphate (Sudakin and Stone 2011), was predictive of enlargement of the cortical surface in several areas including superior and middle temporal gyrus, post-central gyrus, superior frontal gyrus, cuneus and precuneus, and gyrus rectus (Rauh et al. 2012). Furthermore, increased exposure was associated with lower cortical thickness of frontal, temporal, and parietal regions. In contrast to these findings, no evidence of an association with cortical surface area and cortical thickness was observed in our study. The inconsistency in the results may be explained by the differences in OP pesticide exposure, in exposure assessment methodology, or in study populations. Rauh et al. (2012) measured a single OP pesticide chlorpyrifos in cord blood, while in our study we measured DAP metabolites at early-, mid-, and late-pregnancy in urine as a biomarker of OP pesticide exposure. DAP metabolites provide non-specific data about the total exposure to several OP pesticides instead of the exposure to a single OP pesticide. Mothers in this study were most likely exposed to a combination of different OP pesticides that also produce DE metabolites. Of all the insecticides that were applied in 2004 in the Netherlands, 32% were OP pesticides that produces DM metabolites and only 1% were pesticides that generate DE metabolites (CBS 2020). Of the latter, the OP pesticide chlorpyrifos accounted for 1/3 of the total generated DE metabolites. This suggests that exposure to chlorpyrifos may have been lower in our population. However, between 2004 and 2006 OP pesticide residues of chlorpyrifos coming from importation have been detected on tested vegetables and fruits (ChemKap 2017). Of note, DAP metabolite concentrations in this study are higher compared to most other birth-cohort studies (van den Dries et al. 2018). Other

differences between the studies relate to socio-economic status, as the population in the previous study was socially disadvantaged. It is conceivable that in these populations unmeasured background risk factors related to both chlorpyrifos exposure and brain morphology might lead to potential residual confounding.

To our knowledge, this is the first epidemiological study that investigated prenatal exposure to OP pesticides and white matter microstructure. In preadolescents aged 9-12 years, the development of many white matter tracts, such as projection of the prefrontal cortex, is still ongoing (Lebel et al. 2019). Altered maturation of white matter microstructure might therefore result in neurodevelopmental problems with long-term clinical implications. Studies have found that altered white matter microstructure is associated with impaired cognition, behavior problems, and neurodevelopmental disorders (Lebel and Deoni 2018). We observed that increased OP pesticide exposure during pregnancy was associated with lower FA and higher MD of white matter [e.g., a 10-fold increase in averaged DM metabolite concentrations was associated with 1.00 lower FA (95%CI: -1.80, -0.20)], and that the direction of the associations was consistent across most specific tracts. To help interpret these results we calculated the association of child age with white matter microstructure, as age is a robust determinant of the latter. A one-year increase in age was associated with 0.88 (95%CI= 0.35, 1.42) increase in global FA and a 0.10 (95%CI= -0.16, -0.05) decrease in global MD. This implies that, for example, a 10-fold increase in averaged DM concentrations during pregnancy has a similar effect as being 1.1 years younger in terms of white matter microstructure.

Global FA and MD are indicators of white matter microstructure (Alexander et al. 2007). FA describes the propensity for enhanced water diffusion in the white matter tracts whereas MD expresses the scale of average water diffusion in every direction within brain tissue (Alexander

et al. 2007). A lower FA and higher MD can be a result of several reasons including lower packing of axons, higher membrane permeability, disturbance of internal axonal structure, and decreased myelination (Lebel et al. 2019). Animal studies also observed similar associations in white matter microstructure in relationship with exposure to OP pesticides. Prenatally chlorpyrifos exposed guinea pigs had lower FA and higher MD within the corpus callosum and the amygdala and rats postnatal (day 1 until day 6) exposed to chlorpyrifos had a decreased expression of the myelin-associated glycoprotein in the brain which is crucial for the preservation of the mature myelinated unit (Betancourt et al. 2006; Mullins et al. 2015). Moreover, chlorpyrifos exposure reduces the polymerization of tubulin (Grigoryan and Lockridge 2009). Tubulin is an protein which plays an important role in the creation of microtubules which are needed for the preservation of the structural and functional integrity of axons (Grigoryan and Lockridge 2009; Terry 2012). In our study we further found an association between OP pesticide exposure and white matter microstructure specific tracts present in projection, association, limbic system, and callosal fibers. Further work is required to confirm our observed association between prenatal OP pesticide exposure and altered white matter microstructure in children.

We observed that the first and second trimester (<18 weeks, and 18-25 weeks) OP pesticide exposure was driving the associated with lower FA and higher MD. White matter growth starts in early gestation and myelination begins in the second trimester (Dubois et al. 2014; Huang et al. 2009; Rice and Barone 2000). OP pesticide exposure has been shown to disrupt the expression of genes and proteins important for the myelination (Slotkin and Seidler 2007). During the second trimester, the development of white matter is especially dependable on signaling pathways such as extracellular ligands, secreted molecules, and transcriptional regulations (Emery 2010). Thus, OP pesticide exposure may alter the courses of later brain

development by influencing axonal growth adhering and group formation and white matter myelination via gene expression alteration.

This study has several limitations. First, urinary DAP metabolite concentrations provide information regarding the joint exposure to multiple OP pesticides instead of providing specific information regarding the exact OP pesticide exposure (Duggan et al. 2003; Margariti et al. 2007; Wessels et al. 2003). It is therefore unknown to which specific OP parent pesticide(s) our study population was exposed. However, the use of DAP metabolites as biomarkers of OP pesticides is also a strength because it allows for the identification and comparison OP pesticide exposure levels within and between study populations (Bravo et al. 2004). Second, DAP metabolites are characterized by a short half-life and are excreted in urine within one or two days. This implies that the biomarker concentrations may differ from day-to-day within each subject as a consequence of variable contact with exposure sources (e.g., variable diet patterns) and result in (within-subject) temporal variability (Lu et al. 2008; Needham 2005). Although we included 3 urine spot samples which is more frequent that most other studies exploring the association between prenatal OP pesticide exposure and neurodevelopment, it would be preferable to collect more urine specimens during pregnancy to reduce the measurement error and attenuation bias caused by the within-subject variably (Perrier et al. 2016). Third, this study was restricted by the nonappearance of information on possible residential pesticide use by the participant, another household member, or a professional exterminator. Participants in this study might have been exposed through the use of residential products which may contain OP pesticides such as insecticides for the lawn and garden (e.g., emulsifiable concentrate), insecticides for house plants, residential pest products (e.g., fly control insecticides and moth killer cassettes), and flea products for pets. Although the use of products that contain OP pesticides is unlikely to confound the association between prenatal OP pesticide exposure and

brain morphology, such information would be helpful in determining the exact sources of exposure. The Generation R Study is representative of an urban population of which the exposure to OP pesticides most likely occurs through diet (van den Dries et al. 2018). The results of this study may therefore not be fully generalizable to semi-urban and rural areas in the Netherlands where the source of OP pesticides exposure could be different Finally, this study was limited by the absence of information on exposure to other types of pesticides. While we included many possible confounders in our analyses, we cannot eliminate the existence of potential residual confounding in this study as a consequence of unidentified background risk factors that are predictive of OP pesticide exposure and brain development.

The present study has a number of strengths. This study has a large sample size and the availability of potential confounders such as the IQ of the mother and socioeconomic factors. Further, the scanning procedure in which all brains were scanned using the same MRI scanner and software to reduce potential measurement error is another strength.

In conclusion, prenatal OP pesticide exposure was not associated with cortical brain volume, cortical thickness, and cortical surface area in preadolescents aged 9-12 years. However, we found that prenatal OP pesticide exposure was associated with lower FA and higher MD of white matter, and that early and mid-pregnancy were particularly sensitive windows of vulnerability of OP pesticide exposure. These findings suggest that prenatal exposure to worldwide commonly applied OP pesticides may alter normal white matter microstructure development in children, which could have consequences for normal neurodevelopment. Besides structural brain changes, functional brain alteration may also provide opportunities to deepen the understanding of the effects of prenatal exposure to OP pesticides on the brain, as a recent study has done (Sagiv et al. 2019). Future longitudinal studies on brain imaging are

warranted to reproduce our findings, as well as to investigate the mediating role of the structural and functional brain alterations in the association between prenatal exposure to OP pesticides and neuropsychological development. If the findings of his study are confirmed, public health policies that aim towards stricter regulation and control of OP pesticides application should be implemented both in Europe and worldwide.

Acknowledgement

We would like to acknowledge all the partners involved in the Generation R Study which includes the Erasmus Medical Center, Rotterdam, the Faculty of Social Sciences of the Erasmus University Rotterdam, Rotterdam Homecare Foundation and Stichting Trombosedienst & Artsenlaboratorium Rijnmond. Further, the authors sincerely appreciate Dr. Frank Pierik effort for arranging the basis for the NIEHS-TNO-Erasmus University partnership.

References

Alexander AL, Lee JE, Lazar M, Field AS. 2007. Diffusion tensor imaging of the brain. Neurotherapeutics 4:316-329.

Betancourt AM, Burgess SC, Carr RL. 2006. Effect of developmental exposure to chlorpyrifos on the expression of neurotrophin growth factors and cell-specific markers in neonatal rat brain. Toxicological Sciences 92:500-506.

Bradman A, Barr DB, Claus Henn BG, Drumheller T, Curry C, Eskenazi B. 2003. Measurement of pesticides and other toxicants in amniotic fluid as a potential biomarker of prenatal exposure: A validation study. Environmental Health Perspectives 111:1779-1782.

Bravo R, Caltabiano LM, Weerasekera G, Whitehead RD, Fernandez C, Needham LL, et al. 2004. Measurement of dialkyl phosphate metabolites of organophosphorus pesticides in human urine using lyophilization with gas chromatography-tandem mass spectrometry and isotope dilution quantification. 14:249.

CBS. 2020.(Accessed 20 March 2020).

ChemKap. 2017. Agricultural products quality programme database chemkap.

Cook PA, Bai Y, Nedjati-Gilani S, Seunarine KK, Hall MG, Parker GJ, et al. Camino: Open-source diffusion-mri reconstruction and processing. In: Proceedings of the 14th scientific meeting of the international society for magnetic resonance in medicine, 2006, Vol. 2759Seattle WA, USA, 2759.

Costa LG, Giordano G, Guizzetti M, Vitalone A. 2008. Neurotoxicity of pesticides: A brief review. Front Biosci 13:1240-1249.

de Groot M, Ikram MA, Akoudad S, Krestin GP, Hofman A, van der Lugt A, et al. 2015. Tract-specific white matter degeneration in aging: The rotterdam study. Alzheimer's & Dementia 11:321-330.

Dennis EL, Thompson PM. 2013. Typical and atypical brain development: A review of neuroimaging studies. Dialogues Clin Neurosci 15:359-384.

Dubois J, Dehaene-Lambertz G, Kulikova S, Poupon C, Hüppi PS, Hertz-Pannier L. 2014. The early development of brain white matter: A review of imaging studies in fetuses, newborns and infants. neuroscience 276:48-71.

Duggan A, Charnley G, Chen W, Chukwudebe A, Hawk R, Krieger RI, et al. 2003. Di-alkyl phosphate biomonitoring data: Assessing cumulative exposure to organophosphate pesticides. Regulatory Toxicology and Pharmacology 37:382-395.

Eaton DL, Daroff RB, Autrup H, Bridges J, Buffler P, Costa LG, et al. 2008. Review of the toxicology of chlorpyrifos with an emphasis on human exposure and neurodevelopment. Critical Reviews in Toxicology 38:1-125.

Emery B. 2010. Regulation of oligodendrocyte differentiation and myelination. Science 330:779-782.

EU Pesticides database. 2020. https://eceuropaeu/food/plant/pesticides/eu-pesticides-database/public/?event=homepage&language=EN.

Fenske RA, Lu C, Barr D, Needham L. 2002. Children's exposure to chlorpyrifos and parathion in an agricultural community in central washington state. Environmental health perspectives 110:549-553.

Gilmore JH, Knickmeyer RC, Gao W. 2018. Imaging structural and functional brain development in early childhood. Nat Rev Neurosci 19:123-137.

Grigoryan H, Lockridge O. 2009. Nanoimages show disruption of tubulin polymerization by chlorpyrifos oxon: Implications for neurotoxicity. Toxicology and Applied Pharmacology 240:143-148.

Huang H, Xue R, Zhang J, Ren T, Richards LJ, Yarowsky P, et al. 2009. Anatomical characterization of human fetal brain development with diffusion tensor magnetic resonance imaging. Journal of Neuroscience 29:4263-4273.

Julien R, Adamkiewicz G, Levy JI, Bennett D, Nishioka M, Spengler JD. 2007. Pesticide loadings of select organophosphate and pyrethroid pesticides in urban public housing. 18:167.

Kooijman MN, Kruithof CJ, van Duijn CM, Duijts L, Franco OH, van Ijzendoorn MH, et al. 2016. The generation r study: Design and cohort update 2017. European Journal of Epidemiology 31:1243-1264.

Lebel C, Deoni S. 2018. The development of brain white matter microstructure. NeuroImage 182:207-218.
Lebel C, Treit S, Beaulieu C. 2019. A review of diffusion mri of typical white matter development from early childhood to young adulthood. NMR in Biomedicine 32:e3778.
Lu C, Fenske RA, Simcox NJ, Kalman D. 2000. Pesticide exposure of children in an agricultural community: Evidence of household proximity to farmland and take home exposure pathways. Environmental research 84:290-302.
Lu C, Kedan G, Fisker-Andersen J, Kissel JC, Fenske RA. 2004. Multipathway organophosphorus pesticide exposures of preschool children living in agricultural and nonagricultural communities. Environmental Research 96:283-289.
Lu C, Barr DB, Pearson MA, Waller LA. 2008. Dietary intake and its contribution to longitudinal organophosphorus pesticide exposure in urban/suburban children. Environmental Health Perspectives 116:537-542.
Mahajan R, Chandel S, Chatterjee S. 2019. Environmental fate of organophosphate residues from agricultural soils to fresh farm produce: Microbial interventions for sustainable bioremediation strategies. In: Microbes and enzymes in soil health and bioremediation:Springer, 211-224.
Margariti MG, Tsakalof AK, Tsatsakis AM. 2007. Analytical methods of biological monitoring for exposure to pesticides: Recent update. Ther Drug Monit 29:150-163.
Mizuno Y, Kagitani-Shimono K, Jung M, Makita K, Takiguchi S, Fujisawa TX, et al. 2019. Structural brain abnormalities in children and adolescents with comorbid autism spectrum disorder and attention-deficit/hyperactivity disorder. Translational Psychiatry 9:332.
Muetzel RL, Mous SE, van der Ende J, Blanken LME, van der Lugt A, Jaddoe VWV, et al. 2015. White matter integrity and cognitive performance in school-age children: A population-based neuroimaging study. Neuroimage 119:119-128.
Muetzel RL, Blanken LME, van der Ende J, El Marroun H, Shaw P, Sudre G, et al. 2017. Tracking brain development and dimensional psychiatric symptoms in children: A longitudinal population-based neuroimaging study. American Journal of Psychiatry 175:54-62.
Muetzel RL, Mulder RH, Lamballais S, Cortes Hidalgo AP, Jansen P, Güroğlu B, et al. 2019. Frequent bullying involvement and brain morphology in children. Frontiers in Psychiatry 10.
Mullins RJ, Xu S, Pereira EFR, Pescrille JD, Todd SW, Mamczarz J, et al. 2015. Prenatal exposure of guinea pigs to the organophosphorus pesticide chlorpyrifos disrupts the structural and functional integrity of the brain. Neurotoxicology 48:9-20.
Needham LL. 2005. Assessing exposure to organophosphorus pesticides by biomonitoring in epidemiologic studies of birth outcomes. Environmental Health Perspectives 113:494-498.
Perrier F, Giorgis-Allemand L, Slama R, Philippat C. 2016. Within-subject pooling of biological samples to reduce exposure misclassification in biomarker-based studies. Epidemiology (Cambridge, Mass) 27:378-388.
Prieler J. 2003. Raven's advanced progressive matrices. Schufried, Mödling, Austria.
R core Team. 2015. A language and environment for statistical computing. Vienna, austria. R Foundation for Statistical Computing.
Rauh VA, Perera FP, Horton MK, Whyatt RM, Bansal R, Hao X, et al. 2012. Brain anomalies in children exposed prenatally to a common organophosphate pesticide. Proceedings of the National Academy of Sciences of the United States of America 109:7871-7876.
Rauh VA, Margolis AE. 2016. Research review: Environmental exposures, neurodevelopment, and child mental health – new paradigms for the study of brain and behavioral effects. Journal of Child Psychology and Psychiatry 57:775-793.
Rice D, Barone S. 2000. Critical periods of vulnerability for the developing nervous system: Evidence from humans and animal models. Environmental Health Perspectives 108:511-533.
Roy TS, Seidler FJ, Slotkin TA. 2004. Morphologic effects of subtoxic neonatal chlorpyrifos exposure in developing rat brain: Regionally selective alterations in neurons and glia. Developmental brain research 148:197-206.

Roy TS, Sharma V, Seidler FJ, Slotkin TA. 2005. Quantitative morphological assessment reveals neuronal and glial deficits in hippocampus after a brief subtoxic exposure to chlorpyrifos in neonatal rats. Developmental brain research 155:71-80.
Sagiv SK, Bruno JL, Baker JM, Palzes V, Kogut K, Rauch S, et al. 2019. Prenatal exposure to organophosphate pesticides and functional neuroimaging in adolescents living in proximity to pesticide application. Proceedings of the National Academy of Sciences 116:18347.
Sapbamrer R, Hongsibsong S. 2019. Effects of prenatal and postnatal exposure to organophosphate pesticides on child neurodevelopment in different age groups: A systematic review. Environmental Science and Pollution Research 26:18267-18290.
Savy CY, Fitchett AE, Blain PG, Morris CM, Judge SJ. 2018. Gene expression analysis reveals chronic low level exposure to the pesticide diazinon affects psychological disorders gene sets in the adult rat. Toxicology 393:90-101.
Slotkin TA, Seidler FJ. 2007. Comparative developmental neurotoxicity of organophosphates in vivo: Transcriptional responses of pathways for brain cell development, cell signaling, cytotoxicity and neurotransmitter systems. Brain research bulletin 72:232-274.
Slotkin TA, Bodwell BE, Levin ED, Seidler FJ. 2008. Neonatal exposure to low doses of diazinon: Long-term effects on neural cell development and acetylcholine systems. Environmental Health Perspectives 116:340-348.
Steenweg-de Graaff J, Roza SJ, Steegers EA, Hofman A, Verhulst FC, Jaddoe VW, et al. 2012. Maternal folate status in early pregnancy and child emotional and behavioral problems: The generation r study. Am J Clin Nutr 95:1413-1421.
Sudakin DL, Stone DL. 2011. Dialkyl phosphates as biomarkers of organophosphates: The current divide between epidemiology and clinical toxicology. Clinical Toxicology 49:771-781.
Terry AV. 2012. Functional consequences of repeated organophosphate exposure: Potential non-cholinergic mechanisms. Pharmacology & therapeutics 134:355-365.
Textor J, van der Zander B, Gilthorpe MS, Liśkiewicz M, Ellison GTH. 2017. Robust causal inference using directed acyclic graphs: The r package 'dagitty'. International Journal of Epidemiology 45:1887-1894.
Valcke M, Samuel O, Bouchard M, Dumas P, Belleville D, Tremblay C. 2006. Biological monitoring of exposure to organophosphate pesticides in children living in peri-urban areas of the province of quebec, canada. Int Arch Occup Environ Health 79:568-577.
van Buuren S, Groothuis-Oudshoorn C. 2011. Mice: Multivariate imputation by chained equations in r.
van den Dries MA, Pronk A, Guxens M, Spaan S, Voortman T, Jaddoe VW, et al. 2018. Determinants of organophosphate pesticide exposure in pregnant women: A population-based cohort study in the netherlands. International Journal of Hygiene and Environmental Health 221:489-501.
van den Dries MA, Guxens M, Pronk A, Spaan S, El Marroun H, Jusko TA, et al. 2019. Organophosphate pesticide metabolite concentrations in urine during pregnancy and offspring attention-deficit hyperactivity disorder and autistic traits. Environment International 131:105002.
Wessels D, Barr DB, Mendola P. 2003. Use of biomarkers to indicate exposure of children to organophosphate pesticides: Implications for a longitudinal study of children's environmental health. Environmental health perspectives 111:1939-1946.
Whyatt RM, Barr DB, Camann DE, Kinney PL, Barr JR, Andrews HF, et al. 2003. Contemporary-use pesticides in personal air samples during pregnancy and blood samples at delivery among urban minority mothers and newborns. Environmental Health Perspectives 111:749-756.

Supporting information

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

Michiel A. van den Dries, Sander Lamballais, Hanan El Marroun, Anjoeka Pronk, Suzanne Spaan, Kelly K. Ferguson, Matthew P. Longnecker, Henning Tiemeier, Mònica Guxens

Corresponding author:

Mònica Guxens
Barcelona Institute for Global Health – Campus Mar
Doctor Aiguader, 88, 08003 Barcelona.
Email: monica.guxens@isglobal.org

Method S1.

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

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

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

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

Method S2.

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

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

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

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

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

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

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

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

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

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

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

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

d. Additionally adjusted for intracranial volume

Table S3. Adjusted[a] association between averaged log10 transformed maternal concentrations of DAP[b] metabolite concentrations in nmol/g creatinine and brain volumes (*n*=441) and white matter microstructure (*n*=474) assessed at child age 10 years.

	Averaged				<18 weeks				18-25 weeks				>25 weeks			
	B	95%CI			B	95%CI			B	95%CI			B	95%CI		
Brain volumes																
Total brain	10.37	-29.32	to	50.05	-3.22	-28.28	to	21.84	11.75	-17.47	to	40.96	8.70	-19.11	to	36.52
Total gray volume	2.54	-21.23	to	26.31	-2.16	-17.11	to	12.79	4.36	-13.14	to	21.87	2.50	-14.15	to	19.16
Subcortical gray matter	-0.01	-1.82	to	1.80	-0.52	-1.66	to	0.62	0.72	-0.62	to	2.07	-0.03	-1.30	to	1.25
Cerebral white matter	8.04	-9.97	to	26.05	-0.90	-12.34	to	10.54	7.49	-5.75	to	20.73	6.23	-6.40	to	18.85
Thalamus[c]	-0.38	-0.77	to	0.01	-0.12	-0.37	to	0.12	-0.10	-0.39	to	0.19	-0.32	-0.59	to	-0.05
Caudate[c]	0.05	-0.32	to	0.43	-0.01	-0.24	to	0.23	0.09	-0.18	to	0.37	0.00	-0.26	to	0.26
Putamen[c]	0.40	-0.03	to	0.83	0.09	-0.19	to	0.36	0.35	0.03	to	0.67	0.17	-0.14	to	0.47
Pallidum[c]	0.03	-0.13	to	0.18	-0.01	-0.11	to	0.09	0.09	-0.02	to	0.20	-0.03	-0.13	to	0.08
Hippocampus[c]	-0.06	-0.32	to	0.20	-0.08	-0.24	to	0.09	0.07	-0.12	to	0.26	-0.05	-0.23	to	0.13
Amygdala[c]	0.06	-0.08	to	0.19	-0.01	-0.10	to	0.07	0.08	-0.02	to	0.18	0.03	-0.06	to	0.12
Nucleus accumbens[c]	0.00	-0.07	to	0.08	-0.02	-0.06	to	0.03	0.05	-0.01	to	0.10	-0.02	-0.07	to	0.04
Cerebellum cortex[c]	-2.96	-6.85	to	0.92	-0.71	-3.17	to	1.74	-2.38	-5.24	to	0.48	-1.34	-4.07	to	1.38
Cerebellar white matter[c]	-0.16	-1.17	to	0.84	-0.01	-0.65	to	0.63	-0.09	-0.84	to	0.65	-0.15	-0.85	to	0.56
Corpus callosum[c]	-0.06	-0.28	to	0.15	0.00	-0.14	to	0.14	-0.01	-0.17	to	0.15	-0.08	-0.23	to	0.07
White matter microstructure																
Global FA	-1.00	-1.82	to	-0.17	-0.68	-1.21	to	-0.16	-0.62	-1.20	to	-0.04	-0.01	-0.59	to	0.56
Global MD	0.13	0.05	to	0.22	0.05	0.00	to	0.11	0.10	0.04	to	0.16	0.03	-0.03	to	0.09

Abbreviations: DAP total dialkyl phosphate, FA= fractional anisotropy, MD= mean diffusivity

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

b. DAP is the sum of dimethylphosphate, dimethylthiophosphate, dimethyldithiophosphate, diethylphosphate, diethylthiophosphate, and diethyldithiophosphate.

c. Additionally adjusted for intracranial volume

Table S4. The adjusted[a] association between log10 transformed maternal DM[b] metabolite concentrations in nmol/g creatinine and fractional anisotropy and mean diffusivity of twelve white matter tracts (*n*=474).

	<18 weeks			18-25 weeks			Averaged		
	B	95% CI		B	95% CI		B	95% CI	
Fractional anisotropy									
Uncinate fasciculus L	0.001	-0.008 to	0.009	-0.002	-0.011 to	0.007	-0.002	-0.015 to	0.012
Uncinate fasciculus R	-0.003	-0.010 to	0.004	-0.003	-0.011 to	0.005	-0.007	-0.018 to	0.005
Cingulate gyrus part of cingulum L	-0.013	-0.025 to	-0.001	-0.003	-0.016 to	0.011	-0.013	-0.033 to	0.006
Cingulate gyrus part of cingulum R	-0.016	-0.027 to	-0.005	-0.008	-0.021 to	0.005	-0.019	-0.037 to	-0.001
Superior longitudinal fasciculus L	-0.004	-0.010 to	0.002	-0.003	-0.010 to	0.004	-0.004	-0.014 to	0.006
Superior longitudinal fasciculus R	-0.007	-0.014 to	-0.001	-0.010	-0.018 to	-0.003	-0.013	-0.024 to	-0.003
Forceps minor	-0.009	-0.018 to	0.001	-0.004	-0.014 to	0.007	-0.017	-0.032 to	-0.002
Forceps major	-0.003	-0.012 to	0.007	-0.007	-0.018 to	0.003	-0.004	-0.019 to	0.012
Inferior longitudinal fasciculus L	-0.006	-0.012 to	0.000	-0.008	-0.015 to	-0.001	-0.008	-0.018 to	0.002
Inferior longitudinal fasciculus R	-0.004	-0.010 to	0.002	-0.007	-0.014 to	0.000	-0.008	-0.018 to	0.002
Corticospinal tract L	-0.006	-0.011 to	0.000	-0.002	-0.008 to	0.004	-0.009	-0.018 to	0.000
Corticospinal tract R	-0.003	-0.009 to	0.002	-0.003	-0.009 to	0.004	-0.008	-0.017 to	0.001
Mean diffusivity[c]									
Uncinate fasciculus L	0.001	-0.005 to	0.007	0.008	0.002 to	0.015	0.008	-0.002 to	0.018
Uncinate fasciculus R	0.005	-0.001 to	0.011	0.008	0.002 to	0.015	0.015	0.006 to	0.025
Cingulate gyrus part of cingulum L	0.008	0.000 to	0.016	0.014	0.004 to	0.023	0.019	0.005 to	0.032
Cingulate gyrus part of cingulum R	0.007	-0.001 to	0.015	0.005	-0.004 to	0.014	0.010	-0.003 to	0.023
Superior longitudinal fasciculus L	0.005	-0.001 to	0.011	0.011	0.004 to	0.018	0.014	0.004 to	0.024
Superior longitudinal fasciculus R	0.008	0.001 to	0.015	0.012	0.004 to	0.020	0.015	0.003 to	0.027
Forceps minor	0.000	-0.009 to	0.009	0.016	0.006 to	0.026	0.017	0.003 to	0.032
Forceps major	-0.006	-0.024 to	0.013	0.015	-0.006 to	0.036	0.010	-0.020 to	0.041
Inferior longitudinal fasciculus L	0.002	-0.006 to	0.009	0.013	0.004 to	0.021	0.012	0.000 to	0.025
Inferior longitudinal fasciculus R	0.005	-0.004 to	0.013	0.012	0.003 to	0.022	0.016	0.002 to	0.030
Corticospinal tract L	0.003	-0.003 to	0.010	0.003	-0.004 to	0.010	0.007	-0.003 to	0.017
Corticospinal tract R	0.001	-0.004 to	0.006	0.005	-0.001 to	0.011	0.004	-0.004 to	0.012

Abbreviations: DM= dimethyl alkyl phosphates, L= left hemisphere, R= right hemisphere.

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

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

c. Mean diffusivity values were multiplied by 10^9

Table S5. The adjusted[a] association between log10 transformed maternal DE[b] metabolite concentrations in nmol/g creatinine and fractional anisotropy and mean diffusivity of twelve white matter tracts (*n*=474).

	<18 weeks			18-25 weeks			Averaged		
	B	95% CI		B	95% CI		B	95% CI	
Fractional anisotropy									
Uncinate fasciculus L	0.000	-0.006 to	0.006	0.002	-0.005 to	0.009	0.001	-0.009 to	0.011
Uncinate fasciculus R	-0.003	-0.008 to	0.002	0.000	-0.006 to	0.006	-0.005	-0.013 to	0.004
Cingulate gyrus part of cingulum L	-0.008	-0.017 to	0.001	-0.003	-0.013 to	0.008	-0.010	-0.025 to	0.005
Cingulate gyrus part of cingulum R	-0.006	-0.014 to	0.003	0.000	-0.009 to	0.010	-0.006	-0.019 to	0.008
Superior longitudinal fasciculus L	-0.003	-0.007 to	0.002	0.000	-0.006 to	0.005	-0.001	-0.009 to	0.006
Superior longitudinal fasciculus R	-0.004	-0.009 to	0.001	-0.004	-0.010 to	0.001	-0.009	-0.017 to	-0.001
Forceps minor	-0.004	-0.011 to	0.003	-0.005	-0.013 to	0.003	-0.010	-0.022 to	0.001
Forceps major	-0.002	-0.009 to	0.005	0.000	-0.008 to	0.008	0.001	-0.010 to	0.013
Inferior longitudinal fasciculus L	-0.002	-0.006 to	0.003	-0.001	-0.006 to	0.004	-0.005	-0.012 to	0.003
Inferior longitudinal fasciculus R	-0.001	-0.005 to	0.004	-0.001	-0.007 to	0.004	-0.003	-0.011 to	0.005
Corticospinal tract L	-0.006	-0.010 to	-0.002	-0.005	-0.009 to	0.000	-0.011	-0.018 to	-0.004
Corticospinal tract R	-0.005	-0.009 to	-0.001	-0.004	-0.009 to	0.000	-0.010	-0.017 to	-0.004
Mean diffusivity[c]									
Uncinate fasciculus L	-0.001	-0.005 to	0.004	0.001	-0.004 to	0.006	0.002	-0.005 to	0.009
Uncinate fasciculus R	0.003	-0.002 to	0.007	0.001	-0.004 to	0.006	0.006	-0.002 to	0.013
Cingulate gyrus part of cingulum L	0.006	0.000 to	0.012	0.008	0.001 to	0.015	0.012	0.002 to	0.023
Cingulate gyrus part of cingulum R	0.004	-0.002 to	0.010	0.002	-0.005 to	0.009	0.005	-0.005 to	0.015
Superior longitudinal fasciculus L	0.003	-0.002 to	0.008	0.002	-0.004 to	0.007	0.006	-0.002 to	0.014
Superior longitudinal fasciculus R	0.003	-0.003 to	0.008	0.001	-0.005 to	0.007	0.004	-0.005 to	0.013
Forceps minor	0.005	-0.002 to	0.011	0.010	0.002 to	0.017	0.016	0.005 to	0.027
Forceps major	-0.005	-0.019 to	0.009	0.002	-0.014 to	0.018	-0.005	-0.028 to	0.018
Inferior longitudinal fasciculus L	0.004	-0.002 to	0.009	0.006	-0.001 to	0.012	0.010	0.001 to	0.019
Inferior longitudinal fasciculus R	0.002	-0.004 to	0.009	-0.001	-0.008 to	0.006	0.002	-0.009 to	0.012
Corticospinal tract L	0.003	-0.002 to	0.007	0.002	-0.003 to	0.007	0.006	-0.002 to	0.013
Corticospinal tract R	0.000	-0.003 to	0.004	0.001	-0.003 to	0.005	0.002	-0.005 to	0.008

Abbreviations: DE= diethyl alkyl phosphates, L= left hemisphere, R= right hemisphere.

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

b. DE is the sum diethylphosphate, diethylthiophosphate, and diethyldithiophosphate.

c. Mean diffusivity values were multiplied by 10^9

Table S6. The adjusted[a] association between log10 transformed maternal DAP[b] metabolite concentrations in nmol/g creatinine and fractional anisotropy and mean diffusivity of twelve white matter tracts (*n*=474).

	<18 weeks			18-25 weeks			Averaged		
	B	95% CI		B	95% CI		B	95% CI	
Fractional anisotropy									
Uncinate fasciculus L	0.001	-0.008 to	0.010	-0.001	-0.011 to	0.009	-0.002	-0.015 to	0.012
Uncinate fasciculus R	-0.005	-0.012 to	0.003	-0.002	-0.011 to	0.006	-0.008	-0.020 to	0.004
Cingulate gyrus part of cingulum L	-0.015	-0.028 to	-0.002	-0.003	-0.017 to	0.011	-0.014	-0.034 to	0.006
Cingulate gyrus part of cingulum R	-0.016	-0.028 to	-0.004	-0.006	-0.019 to	0.007	-0.016	-0.035 to	0.003
Superior longitudinal fasciculus L	-0.005	-0.011 to	0.002	-0.004	-0.011 to	0.004	-0.004	-0.014 to	0.007
Superior longitudinal fasciculus R	-0.008	-0.015 to	-0.001	-0.011	-0.019 to	-0.003	-0.013	-0.024 to	-0.002
Forceps minor	-0.010	-0.020 to	0.000	-0.004	-0.015 to	0.006	-0.017	-0.033 to	-0.002
Forceps major	-0.002	-0.012 to	0.008	-0.007	-0.018 to	0.004	-0.001	-0.017 to	0.015
Inferior longitudinal fasciculus L	-0.007	-0.013 to	0.000	-0.007	-0.015 to	0.000	-0.008	-0.019 to	0.002
Inferior longitudinal fasciculus R	-0.004	-0.011 to	0.002	-0.006	-0.013 to	0.001	-0.007	-0.017 to	0.004
Corticospinal tract L	-0.006	-0.012 to	-0.001	-0.003	-0.009 to	0.004	-0.010	-0.019 to	-0.001
Corticospinal tract R	-0.005	-0.010 to	0.001	-0.004	-0.010 to	0.003	-0.010	-0.019 to	-0.001
Mean diffusivity[c]									
Uncinate fasciculus L	0.000	-0.006 to	0.007	0.008	0.001 to	0.015	0.008	-0.002 to	0.018
Uncinate fasciculus R	0.006	-0.001 to	0.012	0.008	0.001 to	0.015	0.015	0.005 to	0.025
Cingulate gyrus part of cingulum L	0.010	0.002 to	0.019	0.015	0.006 to	0.025	0.021	0.008 to	0.035
Cingulate gyrus part of cingulum R	0.008	-0.001 to	0.017	0.005	-0.005 to	0.014	0.010	-0.004 to	0.024
Superior longitudinal fasciculus L	0.006	-0.001 to	0.013	0.011	0.003 to	0.018	0.014	0.003 to	0.025
Superior longitudinal fasciculus R	0.009	0.001 to	0.016	0.011	0.003 to	0.020	0.014	0.002 to	0.026
Forceps minor	0.002	-0.007 to	0.012	0.018	0.007 to	0.028	0.021	0.006 to	0.036
Forceps major	-0.008	-0.028 to	0.012	0.014	-0.008 to	0.035	0.007	-0.024 to	0.039
Inferior longitudinal fasciculus L	0.004	-0.004 to	0.012	0.014	0.005 to	0.022	0.015	0.002 to	0.027
Inferior longitudinal fasciculus R	0.006	-0.003 to	0.015	0.010	0.001 to	0.020	0.014	0.000 to	0.028
Corticospinal tract L	0.004	-0.002 to	0.011	0.003	-0.004 to	0.010	0.008	-0.002 to	0.019
Corticospinal tract R	0.001	-0.004 to	0.007	0.005	-0.001 to	0.010	0.004	-0.004 to	0.012

Abbreviations: DAP= Total dialkyl phosphates, L= left hemisphere, R= right hemisphere.

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

b. DAP is the sum of dimethylphosphate, dimethylthiophosphate, dimethyldithiophosphate, diethylphosphate, diethylthiophosphate, and diethyldithiophosphate.

c. Mean diffusivity values were multiplied by 10^9

Table S7. P-value of the interaction between averaged log10 transformed DAP[a] metabolite concentrations in nmol/g creatinine and sex in the adjusted[b] association between averaged log10 transformed maternal concentrations of DAP metabolite and brain volumes (*n*=441) and white matter microstructure (*n*=474).

Brain volumes	P-value for interaction
Total brain	0.052
Total gray	0.065
Subcortical gray matter	0.264
Cerebral white matter	0.072
Thalamus[c]	0.961
Caudate[c]	0.212
Putamen[c]	0.899
Pallidum[c]	0.960
Hippocampus[c]	0.826
Amygdala[c]	0.598
Nucleus accumbens[c]	0.307
Cerebellum cortex[c]	0.757
Cerebellar white matter[c]	0.703
Corpus callosum[c]	0.298

White matter microstructure	P-value for interaction
Global FA	0.241
Global MD	0.610

Abbreviations: DAP= Dialkyl phosphates, FA= fractional anisotropy, MD= mean diffusivity

a. DAP is the sum of dimethylphosphate, dimethylthiophosphate, dimethyldithiophosphate, diethylphosphate, diethylthiophosphate, and diethyldithiophosphate

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

c. Additionally adjusted for intracranial volume

Table S8. Adjusted[a] inverse probability weighted association between averaged log10 transformed maternal concentrations of DAP[b] metabolite concentrations in nmol/g creatinine and brain volumes (*n*=441) and white matter microstructure (*n*=474) assessed at child age 10 years.

	Averaged DAP metabolite concentrations in nmol/g creatinine			
Brain volumes	B	95%CI		
Total brain	14.73	-25.37	to	54.84
Total gray	3.39	-20.77	to	27.54
Subcortical gray matter	0.00	-1.84	to	1.84
Cerebral white matter	11.51	-6.53	to	29.55
Thalamus[c]	-0.50	-0.89	to	-0.11
Caudate[c]	0.08	-0.29	to	0.44
Putamen[c]	0.40	-0.03	to	0.83
Pallidum[c]	0.00	-0.15	to	0.15
Hippocampus[c]	-0.08	-0.33	to	0.18
Amygdala[c]	0.08	-0.05	to	0.22
Nucleus accumbens[c]	0.02	-0.06	to	0.09
Cerebellum cortex[c]	-2.54	-6.45	to	1.36
Cerebellar white matter[c]	-0.15	-1.16	to	0.87
Corpus callosum[c]	-0.02	-0.23	to	0.20
White matter microstructure	B	95%CI		
Global FA	-1.03	-1.86	to	-0.20
Global MD	0.13	0.04	to	0.22

Abbreviations: DAP= Dialkyl phosphates, FA= fractional anisotropy, MD= mean diffusivity

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

b. DAP is the sum of dimethylphosphate, dimethylthiophosphate, dimethyldithiophosphate, diethylphosphate, diethylthiophosphate, and diethyldithiophosphate

c. Additionally adjusted for intracranial volume

Table S9. Adjusted[a] association between averaged log10 transformed maternal concentrations of DAP[b] metabolite concentrations in nmol/g creatinine and brain volumes (*n*=441) and white matter microstructure (*n*=474) assessed at child age 10 years with additional adjustment for maternal fruit and vegetable intake.

	Averaged DAP metabolite concentrations in nmol/g creatinine			
Brain volumes	B	95%CI		
Total brain	11.09	-29.35	to	51.53
Total gray	2.17	-22.00	to	26.35
Subcortical gray matter	0.02	-1.83	to	1.86
Cerebral white matter	9.05	-9.37	to	27.46
Thalamus[c]	-0.35	-0.74	to	0.04
Caudate[c]	0.04	-0.34	to	0.41
Putamen[c]	0.43	-0.01	to	0.86
Pallidum[c]	0.05	-0.10	to	0.21
Hippocampus[c]	-0.08	-0.34	to	0.19
Amygdala[c]	0.05	-0.09	to	0.19
Nucleus accumbens[c]	0.00	-0.07	to	0.08
Cerebellum cortex[c]	-2.58	-6.51	to	1.34
Cerebellar white matter[c]	-0.09	-1.11	to	0.93
Corpus callosum[c]	-0.03	-0.25	to	0.18
White matter microstructure	B	95%CI		
Global FA	-0.96	-1.81	to	-0.11
Global MD	0.14	0.05	to	0.23

Abbreviations: DAP= Dialkyl phosphates, FA= fractional anisotropy, MD= mean diffusivity

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

b. DAP is the sum of dimethylphosphate, dimethylthiophosphate, dimethyldithiophosphate, diethylphosphate, diethylthiophosphate, and diethyldithiophosphate

c. Additionally adjusted for intracranial volume

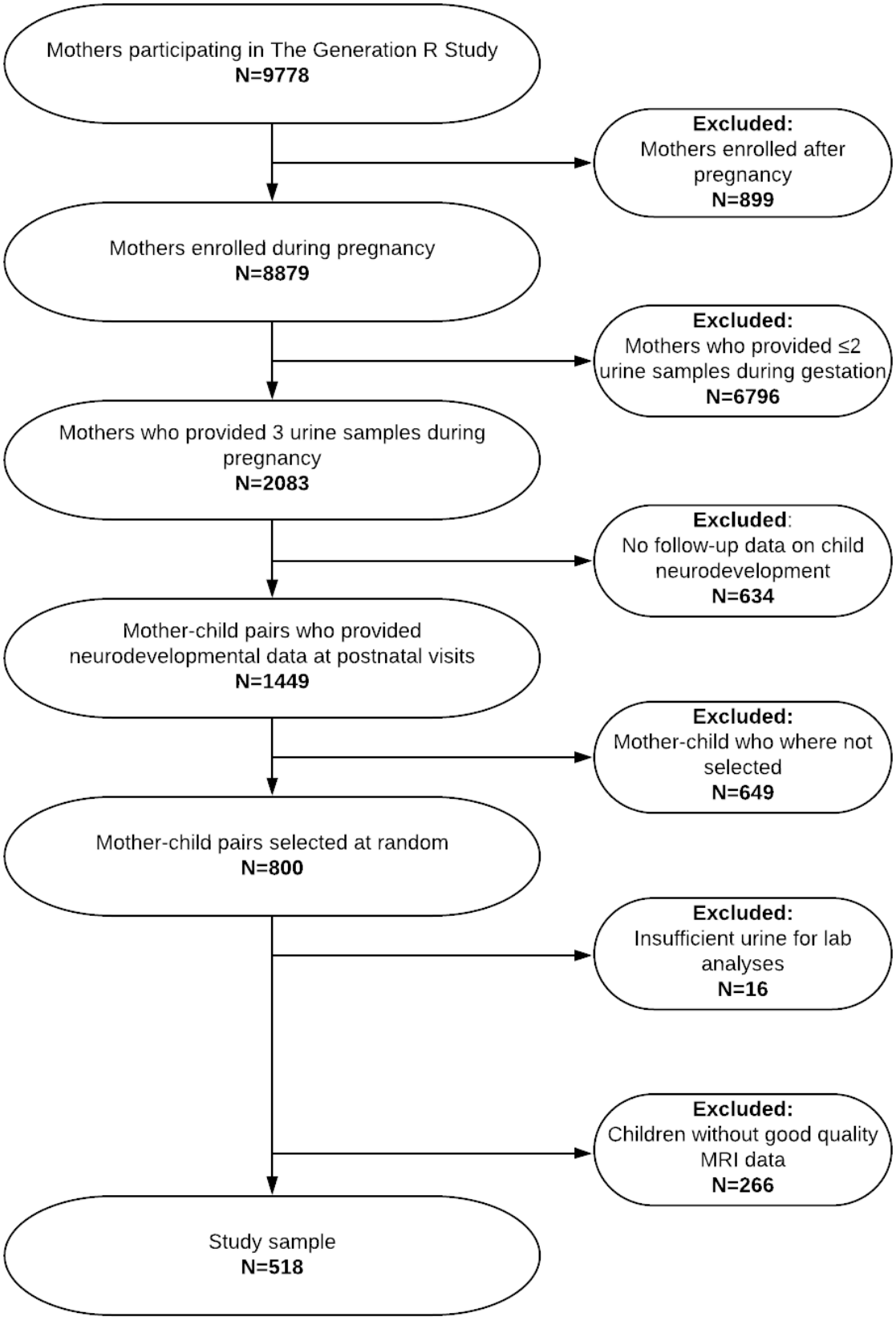

Figure S1. Flowchart of study population.

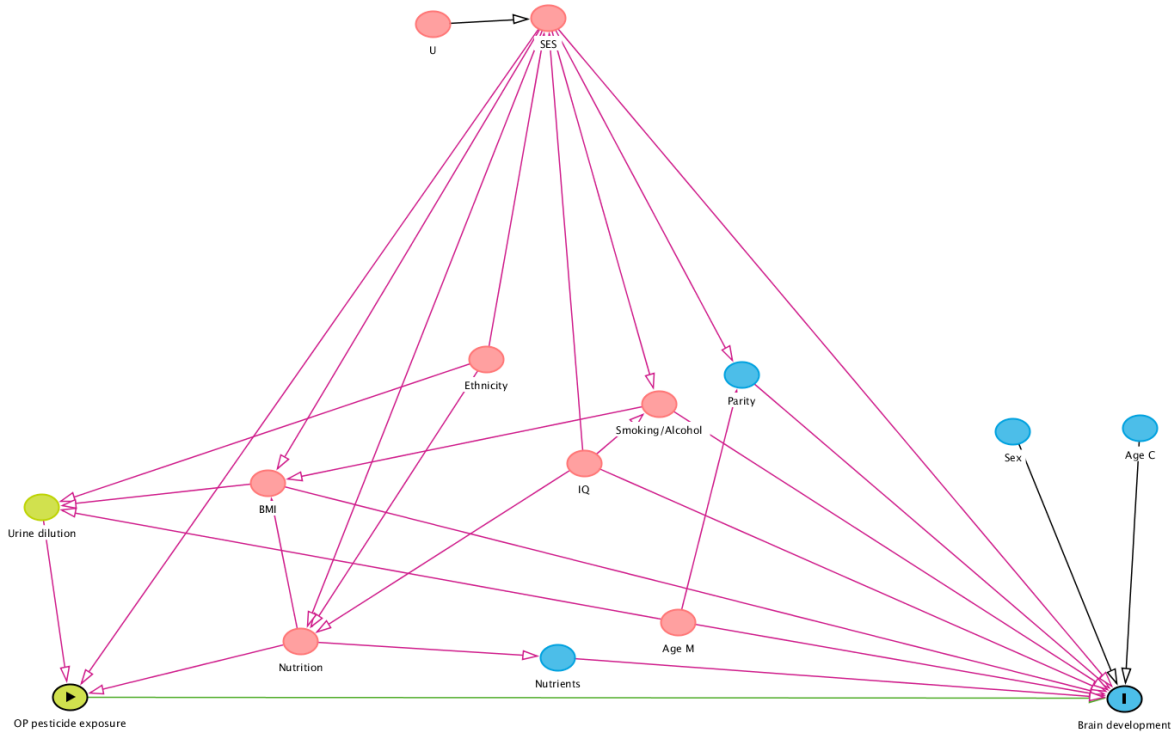

Figure S2. Directed Acyclic Graph of the OP pesticide exposure and brain development. Potential adjustment variables were selected a priori defined with a Directed Acyclic Graph (DAG) using the Dagitty software (Textor et al. 2017). The DAG was based on previous studies of OP pesticide exposure and child neurodevelopment and on biologically plausible covariate–exposure and covariate–outcome associations observed in our data. **Green** circles represent ancestors of the exposure, **blue** circles ancestors of the outcome, **pink** circles ancestors of both exposure and outcome. **BMI**= Maternal body mass index, **SES**= socioeconomic status (maternal education, household income and marital status), **age C**= child age at assessment, **age M**= age mother, **IQ**= maternal nonverbal intelligent quotient, **U**=unobserved ancestor of socioeconomic status.

References

1. Kruithof CJ, Kooijman MN, van Duijn CM, et al. The Generation R Study: Biobank update 2015. *Eur J Epidemiol* 2014; **29**(12): 911-27.
2. Health Canada. Report on Human Biomonitoring of Environmental Chemicals in Canada: Results of the Canadian Health Measures Survey Cycle 1 (2007-2009). In: Health Mo, editor. Ottawa, Ontario; 2010.
3. van den Dries MA, Pronk A, Guxens M, et al. Determinants of organophosphate pesticide exposure in pregnant women: A population-based cohort study in the Netherlands. *International Journal of Hygiene and Environmental Health* 2018.
4. van den Dries MA, Pronk A, Guxens M, et al. Determinants of organophosphate pesticide exposure in pregnant women: A population-based cohort study in the Netherlands. *International Journal of Hygiene and Environmental Health* 2018; **221**(3): 489-501.
5. Butler A. affe Reaction .2. Kinetic Study of Janovsky Complexes Formed from Creatinine (2-Imino-1-Methylimazolidin-4-One) and Acetone. *Journal of the Chemical Society-Perkin Transactions* 1975; **2**: 853-7.
6. van den Dries MA, Guxens M, Pronk A, et al. Organophosphate pesticide metabolite concentrations in urine during pregnancy and offspring attention-deficit hyperactivity disorder and autistic traits. *Environment International* 2019; **131**: 105002.
7. White T, Muetzel RL, El Marroun H, et al. Paediatric population neuroimaging and the Generation R Study: the second wave. *European journal of epidemiology* 2018; **33**(1): 99-125.
8. Fischl B. FreeSurfer. *Neuroimage* 2012; **62**(2): 774-81.
9. Muetzel RL, Mulder RH, Lamballais S, et al. Frequent Bullying Involvement and Brain Morphology in Children. *Frontiers in Psychiatry* 2019; **10**(696).
10. Desikan RS, Ségonne F, Fischl B, et al. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *NeuroImage* 2006; **31**(3): 968-80.
11. Jenkinson M, Beckmann CF, Behrens TEJ, Woolrich MW, Smith SM. FSL. *NeuroImage* 2012; **62**(2): 782-90.
12. Cook PA, Bai Y, Nedjati-Gilani S, et al. Camino: open-source diffusion-MRI reconstruction and processing. 14th scientific meeting of the international society for magnetic resonance in medicine; 2006: Seattle WA, USA; 2006. p. 2759.
13. de Groot M, Ikram MA, Akoudad S, et al. Tract-specific white matter degeneration in aging: the Rotterdam Study. *Alzheimer's & Dementia* 2015; **11**(3): 321-30.
14. Muetzel RL, Blanken LME, van der Ende J, et al. Tracking Brain Development and Dimensional Psychiatric Symptoms in Children: A Longitudinal Population-Based Neuroimaging Study. *American Journal of Psychiatry* 2017; **175**(1): 54-62.
15. Muetzel RL, Mous SE, van der Ende J, et al. White matter integrity and cognitive performance in school-age children: a population-based neuroimaging study. *Neuroimage* 2015; **119**: 119-28.