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Highlights

- Organophosphates (OP) are widely used in agricultural and domestic settings.
- Adverse neuro-outcomes were linked to OP exposure but underpinnings are unknown.
- Functional MRI was performed for 95 children aged 10-12 years.
- Activity in frontal regions decreased with increasing levels of OP metabolites.
- OP may be associated with altered activity in brain regions related to inhibition.

Abstract

Background: Organophosphate pesticides (OP) are widely used for both agricultural and domestic purposes. Epidemiological studies suggest neurotoxicity in children after exposure to organophosphates pesticides (OP) at low levels but possible mechanism is still unclear.

Objectives: We aimed at investigating the effects of prenatal exposure to OPs on inhibitory control of 10 to 12 year-old-children assessed by a motor inhibition task during functional magnetic resonance imaging (fMRI).

Methods: Ninety-five children from the PELAGIE cohort (Brittany-France, from 2002) underwent an fMRI examination during which inhibition was assessed by a Go/No-Go task. Task performance was assessed by average response latency, commission rate and composite performance score (PS). Whole brain activation was estimated by modeling the hemodynamic response related to inhibition demand and successful inhibition. OP exposure was assessed by measuring six dialkylphosphate (DAP) metabolites in the urine of women in early pregnancy (<19 WG). Concentrations were summed to obtain overall levels of diethylphosphate (DE), dimethylphosphate (DM) and total non-specific metabolites (DAP), standardized to homogenize sampling conditions and categorized into levels of exposure: low (reference), moderate or high. Regression models were adjusted for potential cofounders considered by restriction and statistical criteria.

Results: Moderate levels of DAP were associated with a decreased commission rate (β = -6.65%, p = 0.04), indicating improved performance. Increasing levels of DM and DE were associated with decreased brain activity in the left inferior and bilateral superior frontal regions during successful inhibition. We did not observe any differential activation related to inhibitory demands.

Discussion: These results suggest that prenatal OPs may be associated with altered pattern of brain activity in regions related to inhibition among children and need to be confirmed by additional studies.

Keywords :

prenatal exposure, organophosphates, neurodevelopment, motor inhibition, fMRI

Introduction

Organophosphate pesticides (OPs) have been used worldwide since the 1970's for agricultural and pest control purposes due to their high acute neurotoxicity for insects and shorter environmental persistence than that of the organochlorine insecticides they substituted. Their half-life is limited from a few days to several months depending on the environmental conditions. However, they are frequently found, as parent compounds or degradation products, in both outdoor and indoor French environments (Coscollà et al. 2017; Raffy et al. 2017). A common exposure pathway of the general population is thought to be the ingestion of food or drinking water contaminated with organophosphate residues. A study of the typical French diet among pregnant women in 2011 estimated the daily intake of OP residues (including chlorpyrifos) to be sufficient to present a potential risk of neurochemical effects (de Gavelle et al. 2016). Other sources of exposure including the use of household insecticides or living close to areas where OP insecticides are used for agriculture, are very likely in France (Raffy et al. 2017). Since the 2000's, various mixtures of OPs or their metabolites have been frequently found in human biomonitoring studies, in particular in the urine or hair matrices of pregnant French women and children, suggesting widespread and repeated OP exposure (Béranger et al. 2018; Cartier et al. 2016; Dereumeaux et al. 2016).

The primary neurotoxic property of organophosphate compounds for insect control is the inhibition of acetylcholinesterase (AChE) activity in synaptic junctions, resulting in hyper-excitability of the nervous system. Similar effects have also been described in humans after accidental acute exposure. Other neurotoxic mechanisms of OPs have then been described at low doses including non-cholinergic effects, such as oxidative stress, neuro-inflammation, the alteration of axonal transport and mitochondria metabolism, the loss of neurons and glia, and persistent serotonergic effects (Chen et al. 2017; Slotkin and Seidler 2005; Terry 2012).

Low-dose exposure is a matter of concern, especially during prenatal and childhood periods. Biomonitoring studies have reported organophosphate metabolites to be found in the placenta, cord blood and meconium matrices, suggesting the ability of OPs to cross the placental barrier (Silver et al. 2017; Whyatt R M and Barr D B 2001). Given the vulnerability of the developing blood-brain-barrier especially during fetal life (Eskenazi et al. 1999; Gupta et al. 1999; Rice and Barone 2000), the epidemiological literature investigating potential adverse neurodevelopmental effects following prenatal exposure to OPs is extensive.

Although a variety of outcomes has been investigated, several studies have reported adverse effects on specific cognitive and behavioral functions that suggest possible alteration of executive functions (González-Alzaga et al. 2014; Koureas et al. 2012; Muñoz-Quezada et al. 2013). Most of published

studies used neuropsychological tests or standardized questionnaires. We assume that the investigation of functional brain processes while children are engaged in inhibitory cognitive processes, suspected to be affected by OPs, has the potential to provide mechanistic insights into such processes. Task-related functional magnetic resonance imaging (fMRI) is one way to measure cerebral activity during cognitive activity (Horton et al. 2014). A recently published pilot study used functional near-infrared spectroscopy (fNIRS) to investigate neural activity in Mexican-American adolescents from the CHAMACOS cohort (Sagiv et al. 2019). FNIRS is based on the same technique as fMRI, in which changes in neurovascular coupling during cerebral activity are detected. They reported associations between prenatal exposure to OP and altered activation pattern during tasks involving executive functions and language comprehension. There was a bilaterally decreased activity in the inferior frontal cortex during tasks of cognitive flexibility and working memory, which are two executive functions.

Among executive functions, inhibitory control is critical for learning (Bari and Robbins 2013) and difficulties in the ability to control and inhibit a prepotent response can lead to various developmental challenges, such as ADHD (Willcutt et al. 2005) and poor academic performance (Diamond et al. 2007). Our aim was to assess the association between prenatal exposure to OP evaluated by maternal urinary biomarkers, and inhibitory control of 10- to 12- year-old children from a population-based mother-child cohort using fMRI during a motor inhibition task.

Materials and Methods

Study Population

The PELAGIE (*Perturbateurs endocriniens: Etude Longitudinale sur les Anomalies de la Grossesse, l'Infertilité et l'Enfance*) is a population-based mother-child cohort which included 3,421 women before 19 weeks of gestation from three districts of Brittany (France) between 2002 et 2006 (Garlantézec et al. 2009). They were recruited by obstetricians, gynecologists, and ultra-sonographers at early visits for prenatal care. At inclusion, they completed a self-administered questionnaire about family, social, and demographic characteristics, diet, and lifestyle and sent a urine sample to the laboratory. At delivery, midwives and pediatricians at the maternity units provided with information from medical records about the pregnancy, delivery, and neonatal health. Questionnaires given when their children were two and six years old allowed recording further information about the lifestyle, development and health of the child.

A sub-cohort of 251 children between 10 and 12 years of age was randomly selected for the present study to include approximatively 100 children. Children had to be born at term (delivery ≥ 35 weeks

of amenorrhea (WA)) and present no major condition at birth (neonatal hospitalization, hypoglycemia, or five-minute Apgar score < 7), nor prenatal exposure to tobacco, alcohol, or medical treatment during childhood which could affect neurodevelopment (methylphenidate, psychotropic, or antiepileptic drugs, etc.) (Cartier et al. 2016). Among them, 124 (49.4%) families refused to participate or were lost to follow-up, 26 (10.3%) were excluded due to technical (braces) or medical (meningitis, or head trauma requiring medical supervision) reasons. Thus, 101 (40.2%) children participated in the neuropsychological and fMRI examinations. Urine samples were not available for six women because they were used for other urinary assays, resulting in 95 mother-child pairs for our study population (inclusion flowchart is shown in Figure 1).

At the time of follow-up, parents completed a questionnaire about the environmental conditions and health of their child. All parent and child participants provided written informed consent and the appropriate ethics committees approved the study.

Organophosphate insecticide exposure assessment

At inclusion, women returned a urine sample (first morning void) in a 10−mL test tube (95 × 16−mm polypropylene, with wing plug). Six nonspecific dialkylphosphate (DAP), metabolites of numerous OPs were analyzed: dimethylphosphate (DMP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), diethyl-phosphate (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP). Analyses were performed in 2007-2008 (n = 54), 2013 (n = 22), and 2017 (n = 19) by liquid chromatography-electrospray ionization tandem mass spectrometry (LC/ MS-MS) after solid-phase extraction (SPE) at the LABOCEA (Laboratoire public Conseil, Expertise et Analyse en Bretagne). Details of the chemical analysis procedures have been previously described (Cartier et al. 2016).

The limits of quantification (LOQ) for the chemical analyses performed in 2007-2008 (hereafter referred as series n°1) were 1.25, 1.7, 0.02, 0.2, 1, and 0.45 µg/L for DEP, DETP, DEDTP, DMP, DMTP, and DMDTP, respectively. Values between the limit of detection (LOD) and the LOQ were available for series n°2 and n°3 analyzed in 2013 and 2017, respectively. The LODs were 0.2, 0.1, 0.005, 0.06, 0.32, and 0.13 µg/L for DEP, DETP, DEDTP, DMP, DMTP, and DMDTP, respectively. The mass concentrations were converted to molar concentrations using the molecular masses (g/mol) from Toxnet database: DEP (CAS number 598-02-7) 154.101, DETP (CAS number 2465-65-8) 170.168, DEDTP (CAS number 52857-42-8) 186.235, DMP (CAS number 813-78-5) 126.0473, DMTP (CAS number 59401-04-6) 142.114, and DMDTP (CAS number 32534-66-0) 158.181. Concentrations were summed to obtain overall concentrations of diethylphosphate metabolites (DE: DEP, DETP, DEDTP),

dimethylphosphate metabolites (DM: DMP, DMTP, DMDTP), and all nonspecific dialkylphosphate metabolites (DAP).

Motor inhibition function

The Go/No-Go paradigm

The motor inhibition function of the children was evaluated using a 10-minute visual Go/No-Go task completed in the MR scanner. First, a short practice run in front of a computer and then in a mock MR scanner was performed to provide the instructions and accustom the child to the scanner environment.

The event-related task, adapted from Mostofsky et al. (2003), minimizes cognitive demands other than motor execution and response inhibition and can be easily performed in children (Suskauer et al. 2008). Green and red smileys were rapidly presented on a screen, every 1.5 s. Children were asked to press a button as quickly as possible when seeing a green smiley but had to refrain otherwise. The ratio of No-go cues (red smileys) over Go cues (green smileys) was 1:4, to elicit a dominant motor response. The task was implemented using E-Prime v.2.0.8 Professional (Psychology Software Tools, Pittsburgh, PA, USA) and presented using the Nordic Neurolab Solution (Nordic Neurolab, Bergen, Norway).

The task was split into two runs of five minutes each, composed of 150 trials (123 Go and 27 No-Go cues) and four 10-second rest periods a run. A trial consisted of the consecutive presentation of a cue (duration of 200 ms) and a cross fixation point (duration of 1,300 ms). A response was allowed until the next trial began, giving a constant inter-stimulus interval of 1,500 ms. Smileys appeared in a pseudo-random order: cues at the beginning of runs or following resting periods had to be Go cues. Go cues had to occur at least three times in a row and No-Go cues had to occur at most two times in a row.

Performance indicators at the Go/No-Go task

Child performance was evaluated by response latency (average reaction time for the correct answering of Go cues), commission rate (incorrect answers for the No-Go cues), and a composite performance score. Responses occurring during cue presentation (before 200 ms) were considered anticipatory and were excluded from the indicator calculation (on average, less than 1% of all cues). The accuracy of motor inhibition was assessed by the sensitivity index, *d'*, which subtracts the standardized (z score) commission rate from the standardized hit rate (correct answers for Go cues). The response latencies and commission errors inversely correlated with each other (Spearman rho coefficient =-0.31, $p < 0.01$). Thus, in order to combine both response speed and accuracy into a single score, we built a composite performance score by subtracting the response latencies from the

accuracy of motor inhibition (*d'*), following standardization , based on the work of Collignon et al. 2010.

Thus, children with the high scores were considered to efficiently perform the task (fast with a high hit rate, with few commissions), whereas children with the lowest scores were considered to be slow and found it difficult to inhibit their response to No-Go cues. Performance indicators were missing for two children (resulting in n = 93 included children), due to technical issues with the recording.

Cerebral hemodynamic response related to the task

Scanning was performed on a 3 T MR Scanner (Magnetom Verio, VB17, Siemens, Erlangen, Germany) using a 32-channel receiver head coil.

High-resolution 3D anatomical images were obtained using T1-weighted MPRAGE at 1 mm^3 resolution for anatomical referencing (repetition/inversion/echo times: 1,900/900/2.26 ms, 9° flip angle).

Functional images were acquired using gradient echo-planar imaging (EPI) with repetition/echo times of 2,500/30 ms, a 90° flip angle, and 110 volumes per run. Each volume was composed of 34 axially oriented 4 mm-interleaved slices, covering the entire brain. Scans were based on a 110*110 acquisition matrix (220x220 mm^2 field of view), with a voxel size of 2*2*4 mm^3. Operators assessed image quality during the acquisition and two children who moved repeated the sequence. Learning effects are not expected for this task and we did not observe any improvement in performance during the two sessions.

Image pre-processing is described in Supplementary Material. Head movements were evaluated by six parameters calculated during the realignment step. Three children had head motion greater than 2 mm (in-plane size of an acquisition voxel) and they were excluded from further image analysis (resulting in n = 92 included children).

The blood oxygen level dependent (BOLD) response was estimated from trials (Go cue and No-Go cue) and motion regressors, with an implicit baseline corresponding to resting periods (enabling recovery of the hemodynamic response) and the time between trials, using a canonical hemodynamic response function (HRF) and its temporal derivative. This HRF is the subtraction of two gamma functions, the first modelling the peak of intensity, with a latency of 6 s, and the second one the undershoot during the recovery period, with a latency of 16 s. The use of a temporal derivative allows for variations in peak latency, while providing comprehensive models for the response. Scanner drift was modeled with a discrete cosine transform (DCT) set (128-second cut off) and temporal autocorrelation was accounted for using an autoregressive AR(1) model over the entire

brain. First, we modeled Go cues and No-Go cues, independently of the subject's response. Secondly, we modeled successful No-Go, Go, and failed No-Go conditions. Maps were extracted for each condition and each subject by voxel-wise multiple linear regressions estimated by the restricted maximum likelihood (ReML) method and then contrasted. We built two types of individual contrast images, as we were investigating the ability to stop a planned response when it is no longer pertinent. First, we extracted “No-Go vs Go” activations, representing activation amplitudes that were higher for inhibition than motor tasks, when children perceived the inhibition demand. Secondly, we built contrast images for “Successful No-Go vs successful Go”, when children were able to inhibit and stop their answers, expected to reflect successful inhibition. There were no differences between the two runs for contrast estimates (at the uncorrected cluster level $p < 0.001$) or any performance indicators (at $p < 0.05$), allowing concatenation of the two runs.

Statistical analysis strategy

Standardization of sampling conditions and imputation

Sampling conditions of urinary samples of the present study are heterogeneous, in particular duration of storage due to three different chronological series of analyses (2007-2008, 2013 and 2017). Biomarker concentrations may vary according to sampling conditions. Thus, we aimed to standardize the urinary concentrations of DAP under various sampling conditions to limit their possible influence in the association study.

We adapted a standardization method based on regression residuals previously described by Mortamais *et al.* (Mortamais et al. 2012). First, we estimated the influence of sampling conditions (sampling day, duration of storage before and after freezing, and creatinine levels) using an adjusted Tobit regression model to account for multiple censored data. We adjusted the Tobit models for the year and season of sampling, as these parameters may be associated with the *true* level of exposure and reflect temporal variation of OP uses and exposure. Then, left-censored data (values below the LOD) were imputed from a log-normal distribution (Jin et al. 2011). Last, imputed concentrations were standardized using Tobit model estimates as if urines had been sampled under the same conditions (i.e., week-day collection, freezing before 24h after collection, duration of storage < 5 years and median creatinine concentration).

All OP metabolite concentrations used in further statistical analyses were imputed for values below the LOD or LOQ and standardized for sampling conditions (sampling day, duration of storage and creatinine levels) unless specified otherwise. DAP levels were categorized into three groups: for DAP and DM in terciles, and for DE (with > 33% of values < LOD), values < LOD, values > LOD, and < median, values > median. For clarity, we refer hereafter to low, moderate, or high levels of exposure.

Statistical analyses

Multivariable linear regression models were built on performance indicators (log-transformed-response latency, commission rate, and performance score) to analyze the association with each categorical level of OP metabolite. We used restricted cubic splines with log-transformed metabolite concentrations to assess a possible dose-response relation. When checking for the assumption of linearity, we plotted urinary metabolite levels as continuous.

The BOLD response during the motor inhibition task was modeled by mixed effect generalized linear regression models to investigate the effect-modification of the prenatal exposure to OP by the motor inhibition task on cerebral activity. We did not have any *a priori* hypotheses on brain regions that could be differentially activated during the inhibition task across exposure levels and thus performed whole-brain analyses. We then concentrated our interpretation on the regions known to be part of the motor inhibition network (frontal and anterior cingular cortices and supplementary motor area (SMA)) regions (Mostofsky et al. 2003; Suskauer et al. 2008) and also consider other brain regions, such as those than can be involved in ancillary processes (e.g. attention, vision). Statistical significance was assessed for cluster-wise significance, defined by random field theory (FWER corrected $p = 0.05$) to account for 3D spatial autocorrelation, using an uncorrected one-sided cluster-defining threshold of $p = 0.01$ (Nichols and Hayasaka 2003).

Then, we applied our linear regression models to all the children stratified by sex to investigate whether the association between OP metabolite levels and motor inhibition differ according to the sex of the child.

Potential cofounders

Potential adjustment variables were selected from lifestyle and socioeconomic variables collected at inclusion (i.e. maternal age, maternal educational level, pre-pregnancy BMI, pre-pregnancy tobacco consumption, fruit and vegetable consumption and fish consumption during pregnancy), from medical records at delivery (i.e. pregnancy pathologies, child's sex, or during follow-up (i.e. duration of non-exclusive breastfeeding, parity, child's educational level and child's lateralization at follow-up). They were included in all regression models if they were associated (at $p < 0.10$) with both OP metabolite levels and motor inhibition performance (see Supplementary Material, Table S.3); only breastfeeding (≤ vs > 3 months) met the criteria. Fruit and vegetable intake was also forced in all regression models (< *vs* ≥ 3 portions a day), as this has been described to be a possible source of DAP exposure (Sudakin and Stone 2011).

Sensitivity analyses

Two sets of sensitivity analyses were performed. The first one excluded two children with extreme performance scores (statistical outliers outside averaged performance score ± 3* standard errors interval), and the second included OP metabolite levels imputed for values below the LOD but not standardized for sampling conditions.

R software v.3.5.1 (https://www.R-project.org/) and SPM12 (http://www.fil.ion.ucl.ac.uk/spm/) were used for data analysis.

Results

Population characteristics

All pregnant women included in the study were of European origin (Table 1). At inclusion, they had a median age of 30.4 years, were highly educated (46.8% attended at least three years of post-secondary school) and declared to be employed (91.6%). Two thirds declared to be non smokers before inclusion. These women reported few medical problems during pregnancy (3.2% reported high blood pressure, no diabetes). They gave birth mainly to girls (56.8%), with a median birth weight of 3,430 g and 43.2% of the children were breastfed more than three months. Their children at the follow-up time of the present study were mostly right-handed (87.4%), had a median age of 10.8 years, and most attended elementary school (72.6%). We did not observe any differences between the study population and non-participants for these characteristics (see Supplementary material, Table S.1).

Urinary OP metabolite levels

OP metabolites were detected in the urine of 57% of the pregnant women for DE, 86% for DM and 95% for all DAP compounds with respective median values of 0.8, 31.8, and 44.4 µM (see Supplementary material, Table S.2).

Increased creatinine concentrations were associated with increased levels of DE metabolites (1.10^{-4} µM for an increase of one µM of creatinine, $p = 0.02$). A moderate duration of storage before freezing (24-48 h) *versus* a short (< 24h) duration was associated with increased DM metabolite levels ($p < 0.01$) and a moderate duration of storage after freezing (5-10 years) *versus* a short (< 5 years) duration was associated with decreased DM metabolite concentrations ($p < 0.01$). We found no statistically significant association between sampling conditions and DAP metabolite levels nor difference with sampling day (weekday vs weekend) for any OP metabolite (see Supplementary Material, Table S.2). There was an association between analyses series and DAP metabolites ($\beta = -1.0$ for series 2 vs series 1 and $\beta = 0.3$ for series 3 vs series 1, global $p = 0.03$), for DM metabolites ($\beta = -2.6$ for series 2 vs series 1 and $\beta = 0.3$ for series 3 vs series 1, global $p < 0.001$) and not for DE

metabolites (β = 0.06 for series 2 vs series 1 and β = -0.34 for series 3 vs series 1, global p = 0.91) (see Supplementary Material Figure S.I and Table S.2).

As expected, differences did not remain statistically significant after standardization of sampling conditions (for DAP, p = 0.82; for DM, p = 0.23) (see Supplementary Material Figure S.I). The median standardized OP metabolite levels presented were 0.8 µM, 17µM, and 25 µM for DE, DM, and DAP, respectively (see Supplementary Material Table S.3).

Performance indicators and brain activation during the task

Children performed the Go/No-Go task with a median commission rate of 25.9%, and reaction time of 399.6 ms. The performance score ranged from -9.8 to 2.1, with a median of 0.3 (see Supplementary Material Table S.3).

For "No-go vs Go" and "Successful No-Go vs successful Go" contrasts in the brain, children showed activation in regions known to be involved in the motor inhibition network: the anterior cingulate/SMA and inferior and middle frontal regions of both hemispheres. These contrasts were also associated with activated clusters in the right middle temporal region and brain structures of both hemispheres (occipital and parietal region, posterior lobe of the cerebellum, caudate nucleus, and putamen) (see Supplementary Material, Table S.4 and Table S.5).

Prenatal urinary organophosphate metabolite levels and motor inhibition

There was an association between moderate levels of DAP and decreased commission rate (β = -6.65%, 95% confidence interval (CI): -12.83; -0.48), without a statistically significantly higher performance score (β = 0.33, CI: -0.49; 1.15). There was a trend towards a lower performance score (β = -0.76, CI: -1.56; 0.03) and longer response latency (β = $(e^{0.08}-1)*100$ = 8%, CI: 0; 16) associated with moderate DE levels. Children whose mothers had moderate urinary levels of DM metabolites had a lower commission rate (β = -5.59), but the association was not statistically significant (CI: -11.71; 0.53) (Figure 2). Our results did not show a linear dose-response relation between any child's inhibition performance indicators and maternal urinary OP metabolite levels when OP metabolite concentrations were used as continuous variables (Figure 2 and Supplementary material Figure S.II). The associations were similar for children of either sex, with no difference in the estimates, except for commission rate and moderate DE levels (β = 8.23, CI: -0.17; 16.63 for girls (n = 53) and β = -7.33, CI: -15.98; 1.33 for boys (n = 40)).

The first set of sensitivity analyses, which excluded statistical outliers, confirmed the previous statistically significant associations observed between commission rate and moderate DAP levels. Most of the associations were similar in the second set of sensitivity analyses when the OP metabolite levels were not standardized for sampling condition, except that response latency was

significantly higher for children whose mothers showed high DE urinary levels (β = 8%, CI: 0 ; 16) (see Supplementary material Tables S.4 and S.5).

We did not observe any differential activation related to inhibition demand perception. We did not observe any difference in the inhibition network, stratified by sex, except for girls, with decreased brain activity in the medial superior frontal region in association with moderate levels of DM metabolites see Supplementary Material, Table S.6).

When children succeeded in inhibiting their response, there was decreased activation in the left inferior frontal regions associated with high urinary levels of DM metabolites. Children whose mothers had moderate or high levels of DE metabolites exhibited a lower BOLD response in the left and right frontal regions (Figure 3 and Table 2). We did not observe any differential brain activation in girls when stratifying the analyses by sex. However, we observed decreased brain activity in the frontal regions of boys whose mothers had high levels of DAP or DM metabolites or moderate urinary levels of DE metabolites.

Discussion

The present study investigated the potential neurodevelopmental toxicity of prenatal exposure to OPs on motor inhibition function in a Brittany population-based cohort of children. We did not observe any major alterations of the children's performance on the Go/No-Go task. A decreased commission rate associated with moderate levels of DAP suggests better performance. There was no differential brain activation related to inhibitory demands, but we observed decreased cerebral activity related to successful inhibition in regions involved in the motor inhibition network.

Previous epidemiological studies investigating the neurodevelopmental effects of prenatal exposure to OP have suggested possible alterations in children's inhibitory control. In a recent pilot study, Sagiv *et al.* reported an association between prenatal exposure to OPs and altered patterns of brain activity during tasks of executive function and language comprehension using the fNIRS technique on 95 adolescents from a population of agricultural communities of the Salinas Valley in California (CHAMACOS study). They found null associations during the Go/No-Go task but reported decreased bilateral brain activation in the prefrontal cortex during a cognitive flexibility task (Sagiv et al. 2019). In the CHAMACOS study, prenatal exposure to OP was also assessed by two maternal urinary samples collected during the first and second halves of pregnancy. The mothers had higher levels of DAP (109 nM DAP, 17.7 nM DE and 76.8 nM DM) than the present study. The authors reported statistically greater attention problems, ADHD, and K-CPT ADHD Confidence Index scores for five-year-old children (n = 323) and a similar but non-significant association at 3.5 years of age (Marks et al. 2010). Furlong *et al.* investigated the association between prenatal exposure to OP and

neurodevelopmental phenotypes in six- to nine-year-old children (n = 141) at the Mount Sinai Children's Environmental Health Center. Exposure was assessed by analyzing maternal urine collected between 25 and 40 weeks of gestation. Urinary levels were higher than in the PELAGIE cohort (median of 37.1 nM DM and 16.6 nM DE). They reported positive associations between DM metabolite levels and executive functioning factor scores. The impulsivity and externalizing factor score was not associated with maternal urinary levels of DE or DM (Furlong et al. 2017). A cross-sectional study investigated the effect of prenatal and postnatal exposure to OP insecticides on the neurodevelopment of six- to eight-year-old Ecuadorian primary school children (n = 84) and reported a borderline significant association between reaction time and attention test scores and the children's current exposure (Harari Raul et al. 2010). A cross-sectional study evaluated the effects of methyl-parathion (MP) exposure during childhood on six-year-old-children living in Mississippi and Ohio. Exposed children (n=132) were more prone to attention, and behavioral skill problems than controls (n = 147). However, the results were not consistent between the two sites of the study (Ruckart et al. 2004). Although they were not specifically investigating inhibition function, the results of Rauh *et al.* showing that seven-year-old children with high prenatal exposure to chlorpyrifos had frontal cortical thinning, also suggest brain alterations in the frontal cortex, involved in inhibition (Rauh et al. 2012). Findings of brain alterations after prenatal exposure to OPs are supported by one animal study that exposed gestational dams to 5mg/kg/day chlorpyrifos during gestational days 13 to 17. At postnatal day 60, the authors reported reduced neuron and glia count in anterior cingulate, prelimbic, and infralimbic areas of medial prefrontal cortex (mPFC), a region of the inhibition network, at levels showing no effect on body weight, organ coefficient, or pathological morphological changes (such as cytoplasm swelling, enlarged intercellular spaces, or cell apoptosis) in the mPFC (Chen et al. 2017).

Two major types of inhibitory control can be distinguished, cognitive and behavioral inhibition (Bari and Robbins 2013). The task we used involves behavioral response inhibition with a motor component. Response inhibition appears in early childhood and matures until adolescence (Luna and Sweeney 2006). Performance indicators in our population of 10- to 12-year-old children were similar to those reported during a similar Go/No-Go task with 8- to 13-year-old children (median response latency of 400 ms *vs* 407 ms and 25.9% *vs* 22% for commission errors) (Suskauer et al. 2008). We did not observe any differential performance score for the Go/No-Go task but observed decreased brain activity in frontal regions, known to be involved in inhibitory control. Children who are the most highly exposed to OP during pregnancy may present compensatory brain functioning to prevent problems of inhibition. However, we cannot eliminate the possibility that our neuropsychological indicators did not have sufficient sensitivity to discriminate the inhibitory capacity between the

children. In the same way, we cannot rule out that our study (n=95) lacks of statistical power to point out any association, in particular with the sex-stratified analyses (n=41 boys/n=54 girls).

The motor inhibition network concerns three main regions: the anterior cingulate, SMA, and inferior frontal gyri of both hemispheres (Aron 2007; Mostofsky et al. 2003). As expected, we observed cerebral activation in these regions in the contrasts that we explored. We also observed additional activation in other brain structures of both hemispheres (occipital and parietal region, posterior lobe of the cerebellum, caudate nucleus and putamen). These regions are not part of the motor inhibition network but have been described to be involved in certain motor inhibition tasks, in particular in 8- to 13-year-old children (Suskauer et al. 2008). Activation observed in these brain areas may reflect ancillary processes necessary for the Go/No-Go task, such as vision processing, attention and decision making (Behrmann et al. 2004; Grahn et al. 2008; Rosen et al. 2018; Stoodley and Schmahmann 2009). Nevertheless, BOLD imaging measures the ratio of deoxyhemoglobin to oxyhemoglobin to indirectly evaluate neural activity. This indicator is commonly used, but may vary depending on individual characteristics (blood flow, iron deficiency, etc.) and the performance of inhibition is not determined solely by the intensity of the hemodynamic response but could also be explained by brain structure, connectivity, etc. (Ogawa et al. 1990). Reduced activation may indicate that exposure altered the recruitment of neural resources, such as a lack of neural fibers and/or reduced connectivity of the inhibitory network. The associations observed between prenatal levels of DM and DE metabolites and decreased activity from different regions of the brain may suggest an alteration in the pattern of brain activity.

We observed no sex-specific effects on performance indicators but observed decreased brain activity related to successful inhibition only in boys. This finding is consistent with the literature, which suggests that boys may be more sensitive to OP exposure (Comfort and Re 2017; Marks et al. 2010). In their review, Comfort and Re reported that sex-specific effects of OP exposure have been commonly found in rodent studies. Whereas, the exact mechanisms of sex-dependent OP effects are not fully established, authors assumed it could be the combination of altered morphology, neuropeptide and neurotransmitter signaling, and neuroinflammation. They highlighted the necessity to investigate this effect in epidemiological studies. Indeed, Marks *et al.* suggested an increased risk of ADHD at five years of age in the CHAMACOS cohort in association with prenatal exposure to OP in boys only.

Our study was based on a mother-child cohort (PELAGIE study) in which the longitudinal design provides the opportunity to measure OP insecticide exposure during early pregnancy. We were also able to consider lifestyle and socioeconomic factors by restriction or adjustment. In particular,

children whose mothers reported alcohol or tobacco consumption during pregnancy were not included in the study to eliminate two established risk factors of poorer neurodevelopment or behavioral troubles in children (Yolton et al. 2014). We assessed prenatal exposure to OPs by maternal urinary biomarkers, which gives us the ability to capture all sources of exposure. We used fMRI to investigate brain functioning during an inhibition task in association with prenatal exposure to OP in the general population. This technique provides excellent spatial resolution, making it possible to investigate the potential effects of exposure in brain regions of only a few cubic millimeters.

Although we did not find any differences in the characteristics of our study population and non-participants, limiting the risk that a confounder may predict participation, the design of the PELAGIE cohort does not prevent a possible selection bias. Unlike the previously described epidemiological studies, the PELAGIE population does not include an ethnic minority and consists of participants with a high socioeconomic status. We cannot rule out the possibility that the stimulating environments and possible healthier lifestyles in our study population attenuated the potential neurotoxicity of OP exposure. The main limitation of our study was the exposure assessment, based on only one (first) morning void urinary sample collected during the first trimester of pregnancy, whereas OP insecticides have a short half-life, from several hours to several days (Costa 2018). It is likely that our measurement did not capture intra-individual variability, over time, due to the rapid metabolism and excretion of OP insecticides. This produces non-differential measurement errors in the exposure estimation. We could not explore the effects of OP exposure during late pregnancy or childhood, although human neurodevelopment is known to continue during childhood, until adolescence for maturation of the prefrontal cortex (Rice and Barone 2000). A recent study in the Netherlands, showing few effects of prenatal exposure to OP on nonverbal IQ in six-year-old children, suggests that late pregnancy may be a susceptible window for adverse effects on cognition (Jusko et al. 2019). DAP metabolites are found in food and dust. Thus, measurements of urinary levels of DAP may reflect intake of both the toxic parent OP compound and the metabolite itself, without possible distinction and biased exposure classification (Sudakin and Stone 2011).

Conclusion

We found no major alteration of neuropsychological scores in a French population-based cohort of 10 to 12 year-old-children with a high socioeconomic status, but found decreased brain activity in areas related to inhibition in association with prenatal exposure to OPs. These findings need to be confirmed by other studies and more deeply investigated by further neuroimaging techniques of morphometry, and functional or structural connectivity.

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754 Table

Table 1: Characteristics of participants included in the study.

Characteristics	*n=95*	*Study population*
	N	%
Family characteristics		
European origin	95	100 %
Maternal characteristics		
Age		30.4 (28.1;33.5) [a]
<30 years old	41	43.2 %
30-35 years old	40	42.1 %
>35 years old	14	14.7 %
Body Mass Index		
<18.5 kg/m²	10	10.5 %
18.5-25 kg/m²	69	72.6 %
≥25 kg/m²	16	16.8 %
Post-secondary school		
2 years or less	50	53.2 %
≥ 3 years	44	46.8 %
Missing value	1	
Occupation at inclusion		
no	8	8.4 %
yes	87	91.6 %
Smoker before inclusion		
no	62	65.3 %
yes	33	34.7 %
High blood pressure		
no	92	96.8 %
yes	3	3.2 %
Diabetes		
no	94	100 %
Missing value	1	
Fruit and vegetable consumption		
<3 portions/day	78	82.1 %
≥3 portions/day	17	17.9 %
Fish consumption		
< twice a month	62	65.3 %
≥ twice a month	33	34.7 %
Newborn characteristics		
Sex		
Boys	41	43.2 %
Girls	54	56.8 %
Gestational age		
≤39 weeks of amenorrhea	44	46.3 %
>39 weeks of amenorrhea	51	53.7 %
Birth weight		3430 (3165;3800) [a]
<3000 g	12	12.6 %
≥ 3000 g	83	87.4 %
Breastfeeding		2 (0;5.7)
≤ 3 months	54	56.8 %
> 3 months	41	43.2 %
Child characteristics (at follow-up)		
Parity		
single child	16	16.8 %
1 sibling	43	45.3 %
≥ 2 siblings	36	37.9 %
Age		10.8 (10.6;11.1) [a]
10 years old	66	69.5 %

11 years old	29	30.5 %
Educational level		
elementary school	69	72.6 %
junior high school	26	27.4 %
Lateralization		
right-hander	83	87.4 %
left-hander	12	12.6 %

755

756 [a]: median (Q1; Q3)

Table 2: Associations between dialkylphosphate levels in maternal urines during pregnancy and activation intensity related to successful inhibition during the Go/No-Go task of their 10-12 years old children (PELAGIE cohort. 2002-2017) (FWER correction at p<0.05).

Metabolite levels	N	*Right frontal region*		*Left frontal region*		*Anterior cingular region*		*Other brain regions*	
		β (95% CI)	ext.	β (95% CI)	ext.	β (95% CI)	ext.	β (95% CI)	ext.
DAP									
Low levels	31	Ref.		Ref.		Ref.		Ref.	
Moderate levels	28								
High levels	31								
DM									
Low levels	38	Ref.		Ref.		Ref.		Ref.	
Moderate levels	25								
High levels	27			-1.2 (-1.6 ; -0.8)	465				
DE									
Low levels	32	Ref.		Ref.		Ref.		Ref.	
Moderate levels	29	-1.8 (-2.5 ; -1.1)	1072	-1.7 (-2.4 ; -1.0)	951				
High levels	29	-1.6 (-2.0 ; -1.2)	390						

Note: DAP: Dialkylphosphate, DE: diethylphosphate, DM: dimethylphosphate

β (95% IC): estimated coefficient [95% confidence interval]. Empty cell: no statistically significant difference reported in this region.

Estimates are provided for clusters presented in Figure 3. All estimates are from mixed-effect generalized linear regression models adjusted for maternal fruit/vegetable consumption (< 3 portions a day vs ≥ 3 portions a day) and the duration of breastfeeding (< 3 months vs > 3 months).

764 ## Figure legends

Figure 1: Flowchart of inclusions from the PELAGIE cohort (n=3,421) to the present study (n=95).Figure I. Flowchart of inclusions from the PELAGIE cohort (n=3,421) to the present study (n=95).

765 Note: WA: weeks of amenorrhea

766

Figure 2: Associations between OP metabolites levels in maternal urine during pregnancy and performance indicators for the Go/No-Go task of their 10- to 12-year-old children (PELAGIE cohort, 2002-2017) (n =92).

767 Note: DAP: dialkylphosphate, DE: diethylphosphate, DM: dimethylphosphate

768 All estimates are from linear regression models adjusted for maternal fruit/vegetable consumption (<
769 3 portions a day vs ≥ 3 portions a day) and the duration of breastfeeding (< 3 months vs > 3 months).

770 *$p < 0.05$, †: $0.05 \leq p < 0.10$.

771

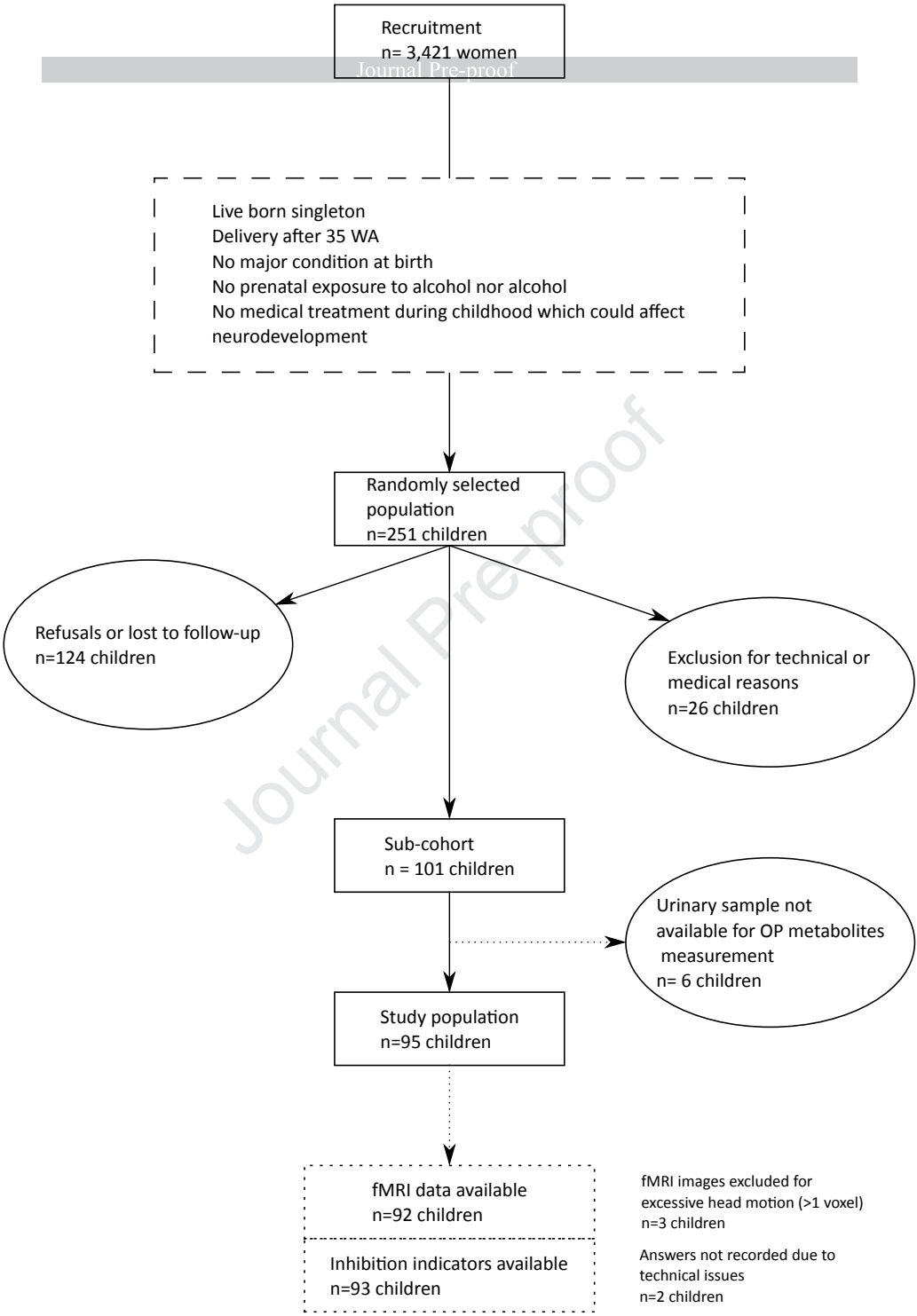
Figure 3: Summary of associations between urinary OP metabolites levels during pregnancy and brain activation during successful inhibition (n = 90)*.

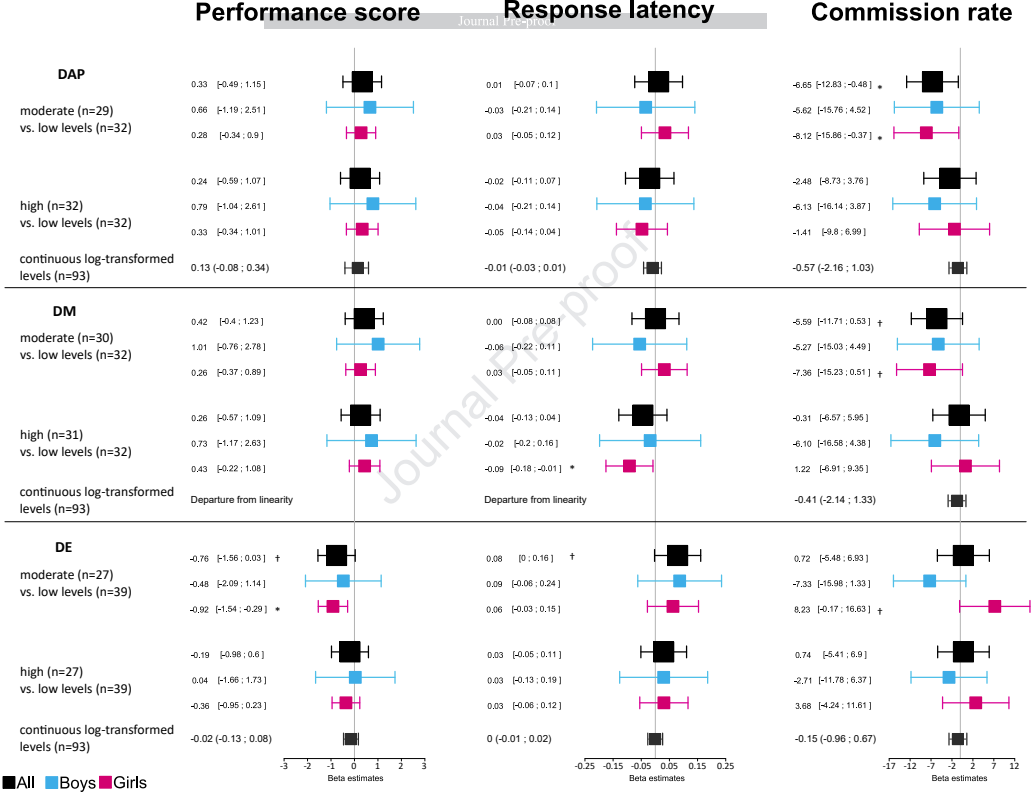
772 Note: DAP: dialkylphosphate, DE: diethylphosphate, DM: dimethylphosphate, inf.: inferior, L: left, R:
773 right

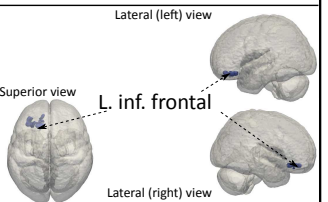
774 Adjusted for maternal fruit/vegetable consumption (< 3 portions a day vs ≥ 3 portions a day) and the
775 duration of breastfeeding (< 3 months vs > 3 months).

776 *FWER corrected-$p \leq 0.05$ with a one-sided uncorrected-$p \leq 0.01$ threshold.

777




Performance score
Response latency
Commission rate
DAP
moderate (n=29) vs. low levels (n=32)
0.33 [-0.49 ; 1.15]
0.66 [-1.19 ; 2.51]
0.28 [-0.34 ; 0.9]
0.01 [-0.07 ; 0.1]
-0.03 [-0.21 ; 0.14]
0.03 [-0.05 ; 0.12]
-6.65 [-12.83 ; -0.48] *
-5.62 [-15.76 ; 4.52]
-8.12 [-15.86 ; -0.37] *
high (n=32) vs. low levels (n=32)
0.24 [-0.59 ; 1.07]
0.79 [-1.04 ; 2.61]
0.33 [-0.34 ; 1.01]
-0.02 [-0.11 ; 0.07]
-0.04 [-0.21 ; 0.14]
-0.05 [-0.14 ; 0.04]
-2.48 [-8.73 ; 3.76]
-6.13 [-16.14 ; 3.87]
-1.41 [-9.8 ; 6.99]
continuous log-transformed levels (n=93)
0.13 (-0.08 ; 0.34)
-0.01 (-0.03 ; 0.01)
-0.57 (-2.16 ; 1.03)
DM
moderate (n=30) vs. low levels (n=32)
0.42 [-0.4 ; 1.23]
1.01 [-0.76 ; 2.78]
0.26 [-0.37 ; 0.89]
0.00 [-0.08 ; 0.08]
-0.06 [-0.22 ; 0.11]
0.03 [-0.05 ; 0.11]
-5.59 [-11.71 ; 0.53] †
-5.27 [-15.03 ; 4.49]
-7.36 [-15.23 ; 0.51] †
high (n=31) vs. low levels (n=32)
0.26 [-0.57 ; 1.09]
0.73 [-1.17 ; 2.63]
0.43 [-0.22 ; 1.08]
-0.04 [-0.13 ; 0.04]
-0.02 [-0.2 ; 0.16]
-0.09 [-0.18 ; -0.01] *
-0.31 [-6.57 ; 5.95]
-6.10 [-16.58 ; 4.38]
1.22 [-6.91 ; 9.35]
continuous log-transformed levels (n=93)
Departure from linearity
Departure from linearity
-0.41 (-2.14 ; 1.33)
DE
moderate (n=27) vs. low levels (n=39)
-0.76 [-1.56 ; 0.03] †
-0.48 [-2.09 ; 1.14]
-0.92 [-1.54 ; -0.29] *
0.08 [0 ; 0.16] †
0.09 [-0.06 ; 0.24]
0.06 [-0.03 ; 0.15]
0.72 [-5.48 ; 6.93]
-7.33 [-15.98 ; 1.33]
8.23 [-0.17 ; 16.63] †
high (n=27) vs. low levels (n=39)
-0.19 [-0.98 ; 0.6]
0.04 [-1.66 ; 1.73]
-0.36 [-0.95 ; 0.23]
0.03 [-0.05 ; 0.11]
0.03 [-0.13 ; 0.19]
0.03 [-0.06 ; 0.12]
0.74 [-5.41 ; 6.9]
-2.71 [-11.78 ; 6.37]
3.68 [-4.24 ; 11.61]
continuous log-transformed levels (n=93)
-0.02 (-0.13 ; 0.08)
0 (-0.01 ; 0.02)
-0.15 (-0.96 ; 0.67)
-3 -2 -1 0 1 2 3
-0.25 -0.15 -0.05 0.05 0.15 0.25
-17 -12 -7 -2 2 7 12
Beta estimates
Beta estimates
Beta estimates
All Boys Girls
Journal Pre-proof

	Low level	Moderate level (vs low)	High level (vs low)
DAP	Reference	*No statistically significant difference*	*No statistically significant difference*
DM	Reference	*No statistically significant difference*	
DE	Reference