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1 Exposure of pregnant women to organophosphate insecticides and child motor
2 inhibition at the age of 10 to 12 years evaluated by fMRI

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17 interests.

18 Highlights

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

24 Abstract

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

28 Objectives: We aimed at investigating the effects of prenatal exposure to OPs on inhibitory control of
29 10 to 12 year-old-children assessed by a motor inhibition task during functional magnetic resonance
30 imaging (fMRI).

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

41 Results: Moderate levels of DAP were associated with a decreased commission rate ($\beta = -6.65\%$, $p =$
42 0.04), indicating improved performance. Increasing levels of DM and DE were associated with
43 decreased brain activity in the left inferior and bilateral superior frontal regions during successful
44 inhibition. We did not observe any differential activation related to inhibitory demands.

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

48 Keywords :

49 prenatal exposure, organophosphates, neurodevelopment, motor inhibition, fMRI

Journal Pre-proof

50 Introduction

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

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

73 Low-dose exposure is a matter of concern, especially during prenatal and childhood periods.
74 Biomonitoring studies have reported organophosphate metabolites to be found in the placenta, cord
75 blood and meconium matrices, suggesting the ability of OPs to cross the placental barrier (Silver et al.
76 2017; Whyatt R M and Barr D B 2001). Given the vulnerability of the developing blood-brain-barrier
77 especially during fetal life (Eskenazi et al. 1999; Gupta et al. 1999; Rice and Barone 2000), the
78 epidemiological literature investigating potential adverse neurodevelopmental effects following
79 prenatal exposure to OPs is extensive.

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

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

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

101

102 Materials and Methods

103 Study Population

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

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

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

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

128 Organophosphate insecticide exposure assessment

129 At inclusion, women returned a urine sample (first morning void) in a 10-mL test tube (95 × 16-mm
130 polypropylene, with wing plug). Six nonspecific dialkylphosphate (DAP), metabolites of numerous
131 OPs were analyzed: dimethylphosphate (DMP), dimethylthiophosphate (DMTP),
132 dimethyldithiophosphate (DMDTP), diethyl-phosphate (DEP), diethylthiophosphate (DETP), and
133 diethyldithiophosphate (DEDTP). Analyses were performed in 2007-2008 (n = 54), 2013 (n = 22), and
134 2017 (n = 19) by liquid chromatography-electrospray ionization tandem mass spectrometry (LC/ MS-
135 MS) after solid-phase extraction (SPE) at the LABOCEA (Laboratoire public Conseil, Expertise et
136 Analyse en Bretagne). Details of the chemical analysis procedures have been previously described
137 (Cartier et al. 2016).

138 The limits of quantification (LOQ) for the chemical analyses performed in 2007-2008 (hereafter
139 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,
140 and DMDTP, respectively. Values between the limit of detection (LOD) and the LOQ were available
141 for series n°2 and n°3 analyzed in 2013 and 2017, respectively. The LODs were 0.2, 0.1, 0.005, 0.06,
142 0.32, and 0.13 µg/L for DEP, DETP, DEDTP, DMP, DMTP, and DMDTP, respectively. The mass
143 concentrations were converted to molar concentrations using the molecular masses (g/mol) from
144 Toxnet database: DEP (CAS number 598-02-7) 154.101, DETP (CAS number 2465-65-8) 170.168,
145 DEDTP (CAS number 52857-42-8) 186.235, DMP (CAS number 813-78-5) 126.0473, DMTP (CAS
146 number 59401-04-6) 142.114, and DMDTP (CAS number 32534-66-0) 158.181. Concentrations were
147 summed to obtain overall concentrations of diethylphosphate metabolites (DE: DEP, DETP, DEDTP),

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

150 Motor inhibition function

151 The Go/No-Go paradigm

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

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

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

171 Performance indicators at the Go/No-Go task

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

181 accuracy of motor inhibition (d'), following standardization, based on the work of Collignon et al.
182 2010.

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

187 Cerebral hemodynamic response related to the task

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

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

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

200 Image pre-processing is described in Supplementary Material. Head movements were evaluated by
201 six parameters calculated during the realignment step. Three children had head motion greater than
202 2 mm (in-plane size of an acquisition voxel) and they were excluded from further image analysis
203 (resulting in $n = 92$ included children).

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

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

224 Statistical analysis strategy

225 Standardization of sampling conditions and imputation

226 Sampling conditions of urinary samples of the present study are heterogeneous, in particular
227 duration of storage due to three different chronological series of analyses (2007-2008, 2013 and
228 2017). Biomarker concentrations may vary according to sampling conditions. Thus, we aimed to
229 standardize the urinary concentrations of DAP under various sampling conditions to limit their
230 possible influence in the association study.

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

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

246 Statistical analyses

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

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

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

266 Potential cofounders

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

277 Sensitivity analyses

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

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

284 Results

285 Population characteristics

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

296 Urinary OP metabolite levels

297 OP metabolites were detected in the urine of 57% of the pregnant women for DE, 86% for DM and
298 95% for all DAP compounds with respective median values of 0.8, 31.8, and 44.4 μM (see
299 Supplementary material, Table S.2).

300 Increased creatinine concentrations were associated with increased levels of DE metabolites (1.10^{-4}
301 μM for an increase of one μM of creatinine, $p = 0.02$). A moderate duration of storage before
302 freezing (24-48 h) *versus* a short (< 24h) duration was associated with increased DM metabolite
303 levels ($p < 0.01$) and a moderate duration of storage after freezing (5-10 years) *versus* a short (< 5
304 years) duration was associated with decreased DM metabolite concentrations ($p < 0.01$). We found
305 no statistically significant association between sampling conditions and DAP metabolite levels nor
306 difference with sampling day (weekday vs weekend) for any OP metabolite (see Supplementary
307 Material, Table S.2). There was an association between analyses series and DAP metabolites ($\beta = -1.0$
308 for series 2 vs series 1 and $\beta = 0.3$ for series 3 vs series 1, global $p = 0.03$), for DM metabolites ($\beta = -$
309 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

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

312 As expected, differences did not remain statistically significant after standardization of sampling
313 conditions (for DAP, $p = 0.82$; for DM, $p = 0.23$) (see Supplementary Material Figure S.I). The median
314 standardized OP metabolite levels presented were $0.8 \mu\text{M}$, $17\mu\text{M}$, and $25 \mu\text{M}$ for DE, DM, and DAP,
315 respectively (see Supplementary Material Table S.3).

316 Performance indicators and brain activation during the task

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

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

326 Prenatal urinary organophosphate metabolite levels and motor inhibition

327 There was an association between moderate levels of DAP and decreased commission rate ($\beta = -$
328 6.65% , 95% confidence interval (CI): -12.83 ; -0.48), without a statistically significantly higher
329 performance score ($\beta = 0.33$, CI: -0.49 ; 1.15). There was a trend towards a lower performance score
330 ($\beta = -0.76$, CI: -1.56 ; 0.03) and longer response latency ($\beta = (e^{0.08}-1)*100 = 8\%$, CI: 0 ; 16) associated
331 with moderate DE levels. Children whose mothers had moderate urinary levels of DM metabolites
332 had a lower commission rate ($\beta = -5.59$), but the association was not statistically significant (CI: $-$
333 11.71 ; 0.53) (Figure 2). Our results did not show a linear dose-response relation between any child’s
334 inhibition performance indicators and maternal urinary OP metabolite levels when OP metabolite
335 concentrations were used as continuous variables (Figure 2 and Supplementary material Figure S.II).
336 The associations were similar for children of either sex, with no difference in the estimates, except
337 for commission rate and moderate DE levels ($\beta = 8.23$, CI: -0.17 ; 16.63 for girls ($n = 53$) and $\beta = -7.33$,
338 CI: -15.98 ; 1.33 for boys ($n = 40$)).

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

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

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

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

356 Discussion

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

363 Previous epidemiological studies investigating the neurodevelopmental effects of prenatal exposure
364 to OP have suggested possible alterations in children's inhibitory control. In a recent pilot study,
365 Sagiv *et al.* reported an association between prenatal exposure to OPs and altered patterns of brain
366 activity during tasks of executive function and language comprehension using the fNIRS technique on
367 95 adolescents from a population of agricultural communities of the Salinas Valley in California
368 (CHAMACOS study). They found null associations during the Go/No-Go task but reported decreased
369 bilateral brain activation in the prefrontal cortex during a cognitive flexibility task (Sagiv et al. 2019).
370 In the CHAMACOS study, prenatal exposure to OP was also assessed by two maternal urinary
371 samples collected during the first and second halves of pregnancy. The mothers had higher levels of
372 DAP (109 nM DAP, 17.7 nM DE and 76.8 nM DM) than the present study. The authors reported
373 statistically greater attention problems, ADHD, and K-CPT ADHD Confidence Index scores for five-
374 year-old children ($n = 323$) and a similar but non-significant association at 3.5 years of age (Marks et
375 al. 2010). Furlong *et al.* investigated the association between prenatal exposure to OP and

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

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

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

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

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

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

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

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

470 Conclusion

471 We found no major alteration of neuropsychological scores in a French population-based cohort of
472 10 to 12 year-old-children with a high socioeconomic status, but found decreased brain activity in
473 areas related to inhibition in association with prenatal exposure to OPs. These findings need to be
474 confirmed by other studies and more deeply investigated by further neuroimaging techniques of
475 morphometry, and functional or structural connectivity.

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753 childhood. *Neurotoxicology and Teratology* 44:30–45; doi:10.1016/j.ntt.2014.05.003.

Table 1: Characteristics of participants included in the study.

Characteristics	n=95 N	Study population %
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/m ²	10	10.5 %
18.5-25 kg/m ²	69	72.6 %
≥25 kg/m ²	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)

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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 cingulate 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						

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

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

760 Estimates are provided for clusters presented in Figure 3. All estimates are from mixed-effect general-
 761 ized linear regression models adjusted for maternal fruit/vegetable consumption (< 3 portions a
 762 day vs \geq 3 portions a day) and the duration of breastfeeding (< 3 months vs $>$ 3 months).

763

764 Figure legends

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

Recruitment
n= 3,421 women
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Live born singleton
Delivery after 35 WA
No major condition at birth
No prenatal exposure to alcohol nor alcohol
No medical treatment during childhood which could affect neurodevelopment

Randomly selected population
n=251 children

Refusals or lost to follow-up
n=124 children

Exclusion for technical or medical reasons
n=26 children

Sub-cohort
n = 101 children

Urinary sample not available for OP metabolites measurement
n= 6 children

Study population
n=95 children

fMRI data available
n=92 children

Inhibition indicators available
n=93 children

fMRI images excluded for excessive head motion (>1 voxel)
n=3 children

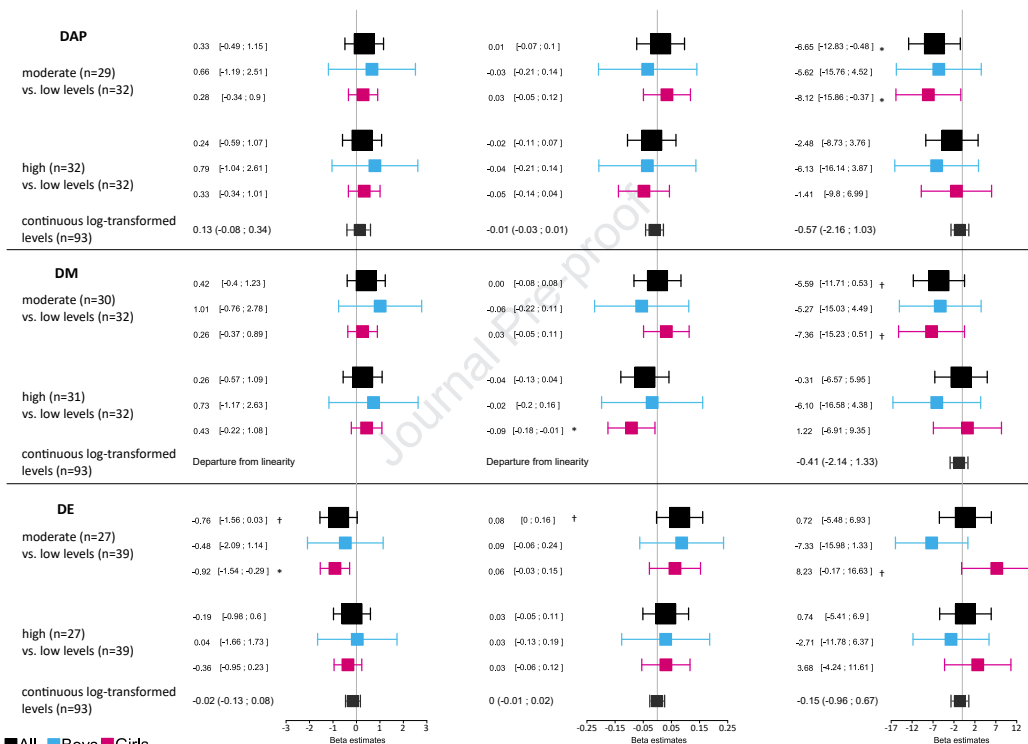
Answers not recorded due to technical issues
n=2 children

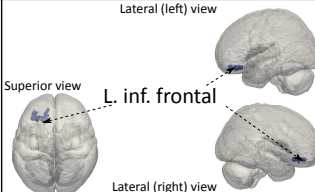
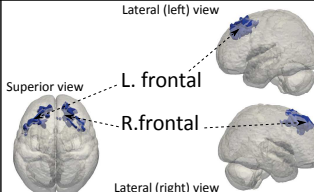
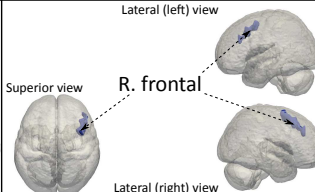
Performance score

Response latency

Commission rate

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	Low level	Moderate level (vs low)	High level (vs low)
DAP	Reference	<i>No statistically significant difference</i>	<i>No statistically significant difference</i>
DM	Reference	<i>No statistically significant difference</i>	 <p>Lateral (left) view Superior view L. inf. frontal Lateral (right) view</p>
DE	Reference	 <p>Lateral (left) view Superior view L. frontal R. frontal Lateral (right) view</p>	 <p>Lateral (left) view Superior view R. frontal Lateral (right) view</p>