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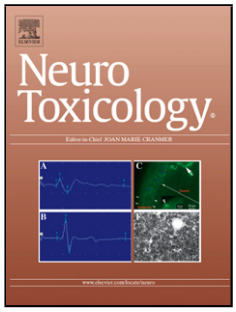

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Children's Contrast Sensitivity Function in Relation to Organophosphate Insecticide Prenatal Exposure in the Mother-Child PELAGIE Cohort

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Highlights

- Very few studies in children have investigated the potential impact of prenatal exposure to organophosphate pesticides (OP) on sensory functions
- The association between urinary OP metabolite levels collected in pregnant women and child's visual contrast sensitivity was evaluated in 180 school-aged children from the mother-child PELAGIE cohort (France)
- Although no associations were observed in the entire sample, maternal OP urinary metabolite levels were associated with a decrease in contrast sensitivity in boys only

Abstract

Human exposure to organophosphate pesticides (OP) is widespread. Several studies suggest that OP prenatal exposure alters the development of cognitive and behavioural functions in children, but the effects of OP prenatal exposure on child sensory functions are largely unknown. The aim of the study was to evaluate the association between OP prenatal exposure and visual processing in school-aged children from the mother-child PELAGIE cohort (France). OP biomarkers of exposure were measured in maternal urine samples at the beginning of pregnancy. The Functional Acuity Contrast Test (FACT) was used to assess visual contrast sensitivity in 180 children at 6 years of age. Linear regression models were performed on all children, and separately for boys and girls, taking into account various potential confounders, including maternal education and breastfeeding. No associations were observed in the whole sample, while maternal OP urinary metabolite levels were associated with a decrease of FACT scores in boys. These findings indicate that OP prenatal exposure might impair visual processing later in life in boys only.

Keywords: Contrast Sensitivity, Vision, Organophosphate Insecticides,Development, Children

1. Introduction

Organophosphate pesticides (OPs) are among the most widely used insecticides worldwide. These chemicals are used for conventional agriculture crops, residential pest control and veterinary purposes. In France, these chemicals have been found in dietary products (Nougadere et al., 2012), outdoor air (Air Breizh 2012), and indoor environments. Human biomonitoring studies have demonstrated widespread contamination (Bradman et al., 2003; Bradman et al., 2013). Indeed OP residues have been found on the hands of children and adults (Bouvier et al., 2006a, 2006b), in urine from different populations, and in amniotic fluid and meconium (Bradman et al., 2003; Whyatt and Barr, 2001; Whyatt et al., 2005). Population exposure to OP has been suggested to occur mainly via dietary intake of non-organic contaminated products (Abb et al., 2010; Lu et al., 2008), but domestic use

of insecticides and proximity to agricultural spraying areas were also found to be potential exposure sources (Whyatt et al., 2005). Breastfeeding and repeated hand-to-mouth behaviours are two other pathways for OP exposure in infants and children (Bedi et al., 2013; Weldon et al., 2011).

OPs were synthesized to eliminate insects through acetylcholine toxicity (Costa, 2006). OPs inhibit acetyl cholinesterase action that results in an increase in acetylcholine levels at synaptic junctions. OPs can also disrupt serotoninergic and dopaminergic pathways, as well as neuronal replication, differentiation and apoptosis during developmental processes (Costa, 2006; Slotkin et al., 2006). OP acute poisoning consequences are well identified but the impact of chronic low dose exposure, as observed in the general population, is still under investigation. Because the brain is more vulnerable to neurotoxic insult during development than later on (Grandjean and Landrigan, 2006; Rice and Barone, 2000), the fetus, newborns and children are potentially the most vulnerable to OP developmental neurotoxicity while prenatal exposure may constitute a window of highest susceptibility.

Prenatal OP exposure has been associated with neurodevelopmental outcomes in several longitudinal birth cohort studies (Gonzalez-Alzaga et al., 2013). Adverse associations in children were observed between prenatal exposure to OP and fine motor skills (Handal et al., 2008), mental and psychomotor development (Engel et al., 2011; Eskenazi et al., 2007; Llop et al., 2013; Rauh et al., 2006), visuospatial performance (Grandjean et al., 2006; Harari et al., 2010), intellectual functions (Bouchard et al., 2011; Engel et al., 2011; Rauh et al., 2011, but see Cartier et al., 2016; Donauer et al., 2016 for contrasting results), as well as symptoms of pervasive developmental disorder (Eskenazi et al., 2010) and attention deficit/hyperactivity disorder (Marks et al., 2010).

Very few studies have investigated the potential impact of prenatal exposure to OP on the integrity of sensory function. This issue needs to be addressed because sensory function is crucial for intellectual or cognitive development, and sensory assessments can potentially reveal brain functional abnormalities that are not detectable with standard cognitive evaluations. The traditional method to assess visual function is acuity testing, which

measures the ability to recognize smaller and smaller stimuli at maximal contrast (e.g., a black letter or any other shape on a white background). Because visual acuity is easily disrupted by refraction errors in the eye and it relies only on high contrast and spatial resolution stimuli, this method may be insensitive to reveal deficits of retinal and/or post-retinal processing, such as the vision loss at low spatial resolution caused by neural defects. Moreover, vision in everyday life is rarely under black-and-white (maximal) contrast. This is why an alternative way to assess vision is through the ability to see a shape or pattern of decreasing contrast (light intensity) against a background. Thus the visual discrimination is tested around the threshold level where the signal (e.g., pattern) is embedded in internal noise (e.g., greater spontaneous neural activity), so that contrast sensitivity represents a discrimination measure of the signal relative to the noise in the visual system (Silvestre et al., 2018). In fact, assessing visual function in the context of signal-to-noise ratio is the key feature of contrast sensitivity testing.

Because contrast sensitivity testing is typically repeated for several stimulus sizes (from large to small), it provides, in comparison to acuity, a more comprehensive and sensitive measure of visual function. This approach has been successfully used to reveal visual neurotoxicity in relation to exposure to several environmental chemicals (e.g., Burbacher et al., 2005; Cartier et al., 2014; Costa et al., 2012; Fillion et al., 2013; Frenette et al., 1991; Indhushree et al., 2016; Jimenez Barbosa et al., 2015; Lebel et al., 1996; Oliveira et al., 2018; Reif et al., 2003; Rice and Hayward, 1999; Saint-Amour et al., 2006; Schreiber et al., 2002; Till et al., 2005). Interestingly, Frenette et al. (1991) have shown that microelectronic workers exposed to solvents have lower contrast sensitivity in comparison to the control group, but normal visual acuity.

To our knowledge, only a single study in children has reported visual function alterations in relation to OP prenatal exposure. Handal et al. (2008) showed that prenatal exposure to OP from maternal occupation in the cut-flower industry during pregnancy was associated with lower visual acuity in Ecuadorian infants and toddlers. However, the OP concentrations were not directly measured in this study, so that the decrease in child acuity might not be associated with prenatal exposure to OP *per se*, but to other flower-industry-

related hazards. The present study aims to examine associations between prenatal OP exposure, measured from OP urinary metabolite samples, and school-age children's visual function, in the French mother-child cohort PELAGIE.

2. Material and methods

2.1. Participants

The PELAGIE cohort was initiated to evaluate the potential impact of prenatal exposure to environmental chemicals on pregnancy outcomes and child development. Between May 2002 and February 2006, 3421 pregnant women from Brittany were recruited before 19 weeks of amenorrhea (Chevrier et al., 2011; Petit et al., 2012; Petit et al., 2010). Questionnaires at inclusion were administered to document social, demographic, occupational, and medical family characteristics, dietary habits (fish, fruit and vegetable consumption), and lifestyle. For urinary assays a subcohort of 601 women (18% of the total cohort) was randomly selected from the cohort members who gave birth to a live-born singleton (Chevrier et al., 2011).

Children at 6 years of age were invited to participate in a neuropsychological follow-up study, that included an evaluation of their contrast sensitivity visual function and cognitive function. Exclusion criteria were the following: birth before 35 weeks of gestation, adverse findings at delivery (e.g., hypoglycemia, 5-minute Apgar score <7), neonatal hospitalization or resuscitation, genetic anomalies, and maternal or child death. Maternal urinary samples and children's vision evaluations were available for 185 of the 243 children who participated in this 6-year-old follow-up study (visual testing material was available for 76.5% of the home visits). Among them 5 children were excluded: 4 children did not wear their glasses at the testing time and one child had nystagmus. All subjects gave informed consent before participating in this study. The appropriate French ethics committees approved all study procedures, including the informed consent forms.

2.2. Measurements of OP exposure

At enrollment, i.e., before 19 weeks of amenorrhea, a first morning void maternal urine sample was collected at home into 10-mL vials containing nitric acid to prevent bacterial multiplication. Pregnant women returned the urine sample to the research laboratory by local mail in a self-addressed stamped package. At reception, samples were frozen at -20 °C until shipment to the analysis laboratory. Children at age 6 were asked to provide the first-morning void urine sample on the day of the home visit, which was retrieved by the psychologist at that time. First morning void urine samples were chosen as the most concentrated urine samples.

Each urine sample, one from the mother and one from the child, was analyzed for six nonspecific dialkylphosphate (DAP) metabolites of numerous OP insecticides (diethylphosphate (DEP), diethylthiophosphate (DETP), diethyldithiophosphate (DEDTP), dimethylphosphate (DMP), dimethylthiophosphate (DMTP) and dimethyldithiophosphate (DMDTP)). The LABOCEA Institute performed the chemical analyses (Plouzané, France). The maternal samples were analyzed by liquid chromatography (Alliance Waters, Separations Module 2690), with a Synergi Fusion RP C18 column, (250 × 2 mm, 4 µm) and triple quadrupole mass spectrometry (LC/MSMS), followed by solid phase extraction (Symbiosis Prospekt II type, Hysphere C18 HD cartridge). Detection relied on LC/MSMS (Quattro Ultima, Micromass/Waters). Two internal standards were used for extraction and detection controls: deuterated diuron 6 and Di-n-butylphosphate, provided by Riedel-de-Haën Fine Chemicals and Dr. Ehrenstorfer GmBH. The children's 1-mL samples were analyzed by solid phase extraction and liquid chromatography (Waters Acquity UPLC), with a Waters BEH C18 column (150 × 2.1 mm, 1.7 µm) and triple quadrupole mass spectrometry (UPLC/MSMS) detection after online solid phase extraction (Waters 2777C and Waters Oasis HLB Direct Connect cartridges). UPLC/MSMS was used for detection (Xévo TQ-S, Waters). Reference standards were di-ethylthiophosphate D10 and dimethylthiophosphate D6 (provided by Sigma-Aldrich and Dr. Ehrenstorfer GmBH). Additional details were provided in the available supplemental data in Cartier et al. (2016).

The limits of quantification (LOQ) for the chemical analyses of maternal urine samples were 1.25, 1.7, 0.02, 0.2, 1, and 0.45 µg/L for DEP, DETP, DEDTP, DMP, DMTP, and

DMDTP, respectively. For the children's samples, values between the limit of detection (LOD) and the LOQ were available. 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. No imputations were made for censored values (i.e., <LOQ for maternal urine samples or <LOD for children's samples). Metabolite concentrations in micrograms per liter were converted to their molar concentrations (nanomoles per liter). DEP, DETP, and DEDTP concentrations were summed to obtain overall concentrations of diethylphosphate metabolites (DE), which are metabolites of the ethyl group of OP (Chlorpyrifos, Diazinon). DMP, DMTP, and DMDTP were summed to obtain dimethylphosphate metabolites (DM), which are metabolites of the methyl group of OP (Dichlorvos, Malathion). DAP were the sum of all six metabolites (sum of DE and DM). The urinary concentrations were then grouped into three exposure categories: 1. censored values, 2. uncensored values ≤ median, 3. uncensored values > median for DE (<66.7% of uncensored values), and in tertiles for DAP and DM (>66.7% of uncensored values).

2.3. Functional Acuity Contrast Test

The Functional Acuity Contrast Test (FACT®) is a chart with five rows of vertical gratings embedded in circular patches (1.7 degrees of visual angle), each having a specific spatial frequency, that is 1.5 cycles per degree (cpd) in row A, 3 cpd in row B, 6 cpd in row C, 12 cpd in row D, and 18 cpd in row E. In each row, the grating contrast uniformly decreases in nine levels by 0.15 log units from high (left side) to low (right side) contrast levels (Fig.1). The FACT was used to assess the children's visual function. Assessments were conducted at home in a normal room illumination by one of two trained psychologists, blind to the child's prenatal and current OP exposure. Ambient light (illumination) was measured just before the test. The child was asked to indicate the grating orientation (left, up or right) for each stimulus. The test was presented monocularly at a distance of 45 cm to the best eye according to the child's preference. The child was instructed to use glasses during the test if normally worn. The child scores, one per row or spatial frequency, were the last correct answer given, so that higher scores indicate better performance. The FACT was administrated twice; a practice session followed by the actual test.

2.4. Potential covariates

A maternal interview was also conducted at home during the 6-year-old follow-up visit, with subtests of the Wechsler Adult Intelligence Scale (WAIS-III) to document the mothers' verbal intellectual quotient (IQ). The Home Observation for Measurement of the Environment questionnaire (HOME) (Caldwell and Bradley, 1979) was also used to assess the family environment.

Several variables were documented with questionnaires at enrollment: Mother's age, education level (university level or lower), tobacco use (yes or no) and alcohol use (yes or no) during pregnancy, fish consumption during pregnancy (≥ 2 per week or less), gestational age, parity (0 or ≥ 1). Additional variables were documented at the 6-years neuropsychological follow up: Mother verbal IQ, HOME score, breastfeeding (yes or no), duration of video gaming (0, 0–1.5, ≥ 1.5 h per week), duration of television watching (<2.5, 2.5–4.5, >4.5 h per week), extra-curricular sports activities (no, yes), sleep duration (10.5, 10.5–11, >11 h per day), the child's examiner (psychologist 1 or 2), and ambient light intensity (in lux units) at the time of testing.

2.5. Statistical analysis

We used linear regression models to assess the relation between maternal urinary concentrations of OP metabolites and children's FACT scores (row A-lowest spatial frequency to row E-highest spatial frequency). The DAP, DM, and DE metabolites were considered in three separate independent models. All variables associated with both prenatal OP metabolite urinary concentrations and FACT scores at $p \leq 0.2$ were considered as potential confounding factors and included in the final regression model (i.e., maternal education level, child's sex, breastfeeding, and duration of television watching). The variables highly associated ($p \leq 0.05$) with FACT scores only were also included in the final regression models (i.e. total HOME score, ambient light, extra-curricular sports activities, and child's interviewer). To account for the potential role of current OP exposure on FACT scores, the children's OP metabolite urinary concentrations were entered in final

regression models. Analyses were conducted for boys and girls separately, but also for the whole sample. Although testing ambient light was optimal for most of children (see Table 1), the range was from 20 to 7880 lux due to various conditions in which the test was administrated at home (room with or without windows, etc.). Because low lighting testing may reduce visual performance, a sensitivity analysis was conducted by re-running the regression models without participants for which the FACT was conducted in non-optimal lighting conditions. All analyses were performed with SPSS software (version 20).

3. Results

Descriptive characteristics of the participants are presented in Table 1. Girls and boys were equally represented in the sample (48.9% and 51.1%, respectively). Mothers' age at the time of study inclusion ranged from 22 to 44 years and most (65.6%) were highly educated (university level). Occasional alcohol drinking (at least once a week) was reported by 12.8% of the pregnant women, and 23.3% reported smoking at the beginning of pregnancy. The average gestation age was 39.5 weeks (median = 40 weeks) with a range of 36 to 41 weeks. Finally, a majority of children (65%) were breastfed, but less than 6 weeks for most of them.

Urinary concentrations of DAP metabolites are presented in Table 2. DAP, DM, and DE metabolites were quantified in 91.7%, 90%, and 49.4% of the prenatal maternal urinary samples, respectively. Among quantified values median urinary concentrations were 47.9 nmol/L for DAP, 39.45 nmol/L for DM, and 13.3 nmol/L for DE. DAP, DM, and DE metabolites were detected in 81.1%, 63.9% and 53.9% of the children's urinary samples, respectively. Median urinary concentrations among detected values were 21 nmol/L for DAP, 15.2 nmol/L for DM, and 10.8 nmol/L for DE. These values are lower than DAP urinary concentrations measured in other North America longitudinal birth cohorts (Cartier et al., 2016).

Descriptive characteristics of FACT scores are presented in Table 3, for all children and for girls and boys separately. Scores did not differ between sex, except for row E where boys had statistically significant better scores than girls (2.84 vs. 2.16, p value = 0.04).

Associations between maternal OP urinary metabolite levels assessed during pregnancy and children's 6-year FACT scores were evaluated using linear regression models for boys and girls separately. When considering boys only, prenatal urinary DE concentrations were significantly associated with a decrease in FACT scores with the highest category level at 6 cpd (<LOQ=ref; β(LOQ-13.3 nmol/L)=-0.63, 95% CI: -1.64; 0.38; β(>13.3 nmol/L)=-1.38, 95% CI: -2.48; -0.29) and 12 cpd (<LOQ=ref; β(LOQ-13.3 nmol/L)=0.06, 95% CI: -0.91; 1.03; β(>13.3 nmol/L)=-1.44, 95% CI: -2.48; -0.39). Moreover, a significant negative association between prenatal urinary concentrations of DAP and FACT scores was found at 12 cpd (<27.7 nmol/L=ref; β(27.7-74.35 nmol/L)=-1.04, 95% CI: -2.05; -0.03; β(>74.35 nmol/L)=-0.44, 95% CI: -1.45; 0.57) (Figure 2). Among girls, almost all estimates were positive and displayed no monotonic dose-response relationship. Statistically significant estimates were observed between intermediate prenatal urinary concentrations of DAP and FACT scores at 3 cpd (β=0.88, 95% CI: 0.02; 1.74) and 6 cpd (β=0.88, 95% CI: 0.03; 1.72), as well as between intermediate prenatal urinary concentrations of DM and FACT scores at 6 cpd (β=0.93, 95% CI: 0.16; 1.69) and 12 cpd (β=1.11, 95% CI: 0.18; 2.04) (Figure 2). No statistically significant association was observed between prenatal total DAP, DE or DM urinary levels and FACT scores for all children as a whole (see Table 4, supplement material).

When considering child OP exposure, no significant association was found between OP urinary metabolite concentrations (DAP, DE, DM) measured at 6 years of age and FACT scores for boys (Figure 3). Significant associations were found for girls between DM urinary concentrations and better FACT scores for the mid-range spatial frequency 6 cpd (<5.24 nmol/L=ref; β(5.24-29.75 nmol/L)=0.66, 95% CI: -0.07; 1.38; β(>29.75 nmol/L)=0.86, 95% CI: 0.85; 1.64) and for the highest spatial frequency 18 cpd (<5.24 nmol/L=ref; β(5.24-29.75 nmol/L)=1.09, 95% CI: 0.12; 2.05; β(>29.75nmol/L)=1.08, 95% CI: 0.05; 2.11) (Figure 3). No association was found for the whole sample (see Table 5, supplement material).

A sensitivity analysis was conducted by re-running the regression models without participants for which the FACT was conducted in low light conditions. Children who was

not tested with at least 55 lux (n=24), which is about the minimal illumination level for photopic or daylight vision, were thus excluded for this analysis. Results revealed no change in the associations reported above (data not shown).

4. Discussion

To our knowledge the present study is one of the first to assess the relation between OP prenatal exposure and visual function in school-aged children using urinary biomarkers of exposure and standardized visual tests in the general population. After controlling for confounding factors no association was observed between maternal DAP, DM or DE urinary levels and children's FACT scores. In boys, prenatal DE levels were significantly associated with a decrease in contrast sensitivity scores in moderate-to-high spatial frequencies (6 and 12 cpd), while better contrast sensitivity scores to the same spatial frequencies were observed among girls in association with prenatal DM urinary concentrations with a non-monotonous relation.

Alterations of visual functions in relation to exposure to other toxic agents have been reported in several human studies, in particular for solvents. In a population of 182 adult workers exposed to organic solvents, Gong et al. reported significantly lower contrast sensitivity than those in the control group but only at spatial frequencies of 6 and 12 cpd; no significant differences were found at 1.5, 3 or 18 cpd. The sex of the subjects was not detailed in this article. In regards to prenatal exposure, gestational exposure to tetrachloroethylene from drinking water has also been associated with lower FACT scores at intermediate and high spatial frequencies in adults, although a statistically significant difference was found only at 18 cpd (Getz et al., 2012). In children, Till et al. (2003) showed, through electrophysiological measures, abnormal chromatic responses and reduced contrast sensitivity in a 2.5-year-old boy following prenatal exposure to tetrachloroethylene. In a subsequent study, on 21 infants born to women who were occupationally exposed to solvents during pregnancy (Till et al., 2005), similar results were observed. That is, in comparison to age-matched controls, significantly lower visual acuity, abnormal chromatic responses (red-green vision) and significantly reduced contrast sensitivity scores in the low and intermediate spatial frequency range (< 10 cpd).

Our findings are in line with previous studies investigating the association between prenatal exposure to OP and visual function. In a cross-sectional study in Ecuador, prenatal occupational exposure to OP pesticides during pregnancy was evaluated through maternal self-reported questionnaires and linked to the visual acuity of the infants and young children (Handal et al., 2008). Visual acuity deficits were found in children from 9 months to 8 years of age whose mothers were occupationally exposed to pesticides during their pregnancy, compared to children whose mothers were not. The authors reported that in these local greenhouses, OP insecticides are the most widely used pesticides, suggesting that prenatal exposure to OP might play an adverse role in children's visual function integrity, as reported in the present study. Of note, recent data collected from a small number of 9-month-old infants in China (n = 27) suggest slower auditory brainstem processing in relation to prenatal exposure to pesticides (Sturza et al., 2016), suggesting that the toxicity of OP on sensory function may not be restricted to the visual modality.

It is generally thought that deficits in contrast sensitivity observed at low-to-medium spatial frequencies mainly reflect defects in post-retinal neural processing, whereas those at high frequencies (and therefore fine spatial vision) are more likely due to the quality of the optics of the eye (Waksman and Brody, 2007). The OP-related reduced scores of contrast sensitivity observed in boys occurred for medium (6-12 cpd) spatial frequency gratings, suggesting alterations of neural processing along the visual pathway. Unexpectedly, better performances of contrast sensitivity in association with intermediate levels of prenatal DM urinary concentrations were observed in girls. We have no explanation for these results, although we cannot exclude the possibility of remaining confounding factors or chance findings. However, sex-dependent effects have been previously observed in studies investigating child neurodevelopment in relation to OP exposure. In a rural California longitudinal birth cohort, prenatal OP exposure was associated with child behavioural problems with a stronger association among boys (Marks et al., 2010). Similarly, memory deficit was observed only in boys in association with prenatal exposure to OP in another North American longitudinal cohort from New York, with no apparent deficit in girls (Horton et al., 2012). Because genetic and hormonal systems differ between boys and girls,

these factors could have played a role in such gender-related differences in OP developmental neurotoxicity.

Our results suggest the importance of assessing the functional integrity of sensory processing when evaluating chemical neurodevelopmental toxicity. Since visual processing precedes cognitive and behavioural response processing, it is possible that neuropsychological performance decrements observed by previous studies in children exposed to OPs are not fully explained by alterations of cognitive processes occurring in high-level brain areas, but may also result, at least in part, from OP sensory alterations. Visual assessment in epidemiological cohort studies, in addition to neuropsychological testing, allow adjustments for potential visual deficits and, therefore, a better prediction of cognitive dysfunction in association with exposure, as shown in the Faroese children in relation to mercury exposure (Grandjean et al., 2001). Moreover, evaluating sensory processes might also reveal subtle functional changes in the brain that are not necessarily detectable with cognitive evaluations.

One major limitation of our study concerns OP exposure assessment. OPs are characterized by a short biological half-life and are rapidly metabolized and excreted in urine (Needham, 2005). A single urine sample as used here is more likely to represent recent OP exposure than chronic cumulative exposure; repeated urinary measures might have enabled better characterization of OP exposure, especially among children (Bradman et al., 2013; Griffith et al., 2011). Moreover, the existence of preformed DAP residues found in food reduces the specificity of our exposure assessment, which makes it impossible to distinguish between exposure to the active parent compound from exposure to the inactive metabolite itself in urinary DAP concentrations (Lu et al., 2008; Zhang et al., 2008). Indeed measurement of DAP residues in fruits and vegetables showed more preformed OP metabolites than parent compounds in the majority of the samples tested (Zhang et al., 2008). Despite these limitations, a urinary biomarker is a simple, noninvasive tool for assessing prenatal OP exposure, and has been shown, in previous studies, to be effective in detecting OP developmental neurotoxicity (Bouchard et al., 2011; Engel et al., 2011; Rauh et al., 2011). Our results are also limited by sample size, in considering statistical analysis

according to child sex; and we also cannot exclude the possibility of chance findings particularly for DE which had the highest frequency of non-detects. Because FACT administration took place at the children's homes, natural variability in the illumination conditions was observed. This variation in illumination was controlled for in our regression models. Nonetheless in order to reduce measurement errors in future studies and to have a better estimate of the influence of OP on CS function it would be helpful to conduct assessments in the same illumination condition for all children using an ophthalmic light for instance.

5. Conclusion

In conclusion, our study showed associations between prenatal biomarkers of OP exposure and children's FACT scores, pointing out the relevance of using standardized visual assessments in cohort studies to better characterized the impact environmental contaminant on child's visual integrity. Replication of these findings is required and further epidemiological and laboratory studies are necessary to make reasonable causal inferences between OP exposure and visual function.

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Conflict of interest statement:
The authors declare that they have no conflict of interest.

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Figure captions

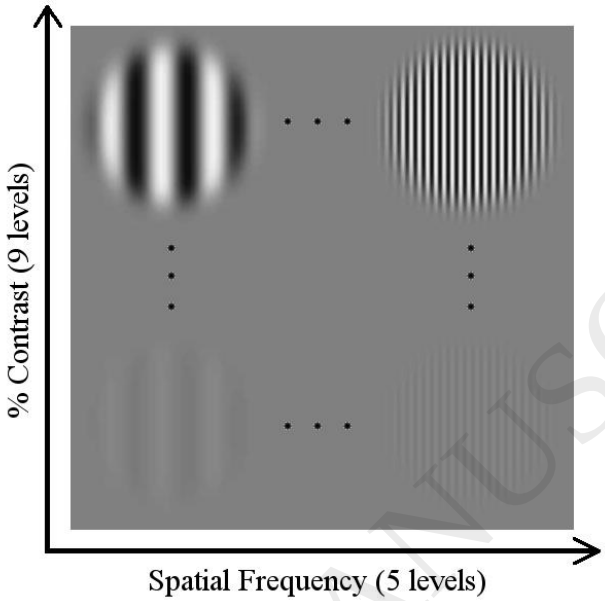

Figure 1. Stimulus changes in terms of contrast (y axis) and spatial frequency (x axis) used in the FACT test.

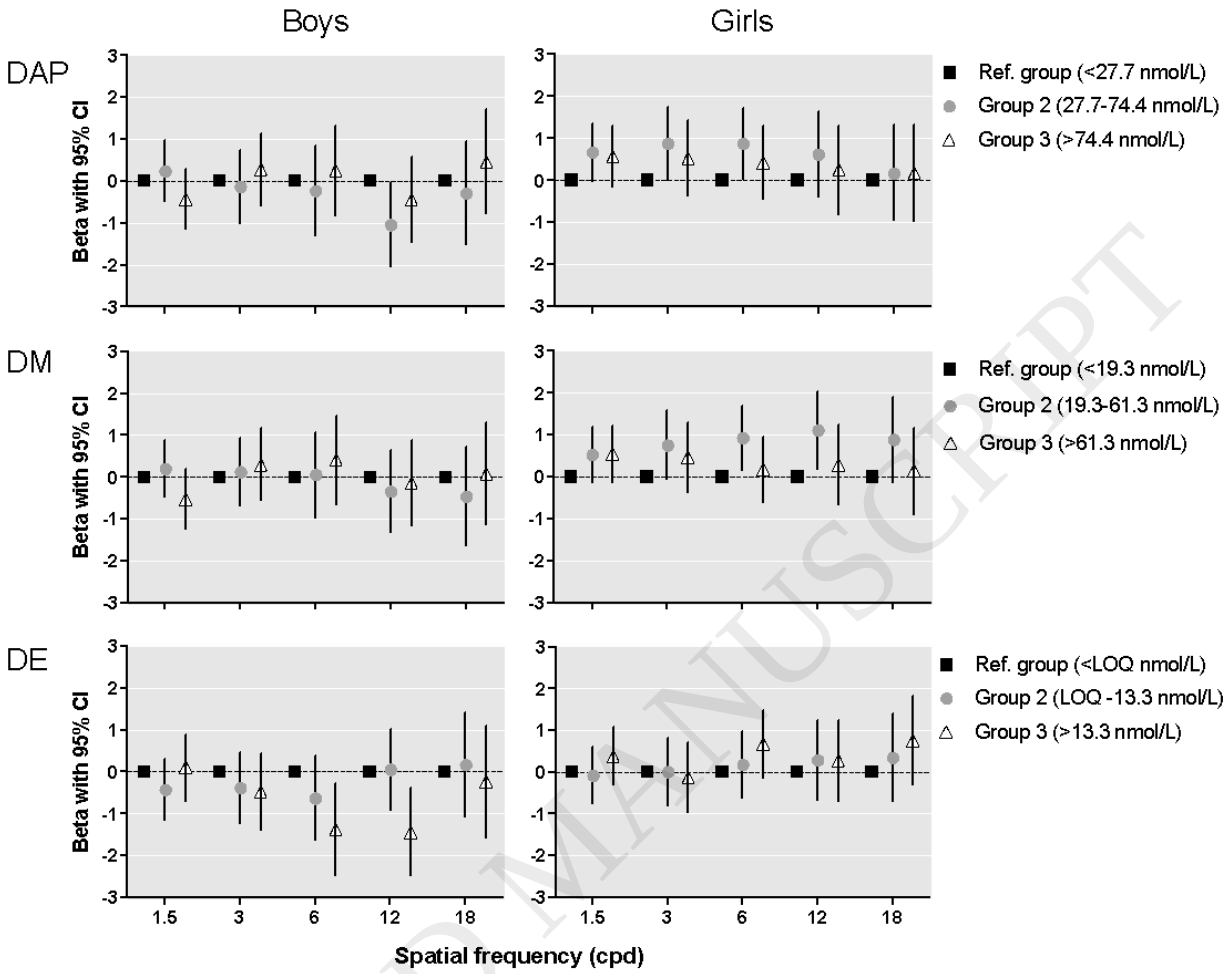

Figure 2. Associations between OP prenatal urinary metabolites and FACT scores.

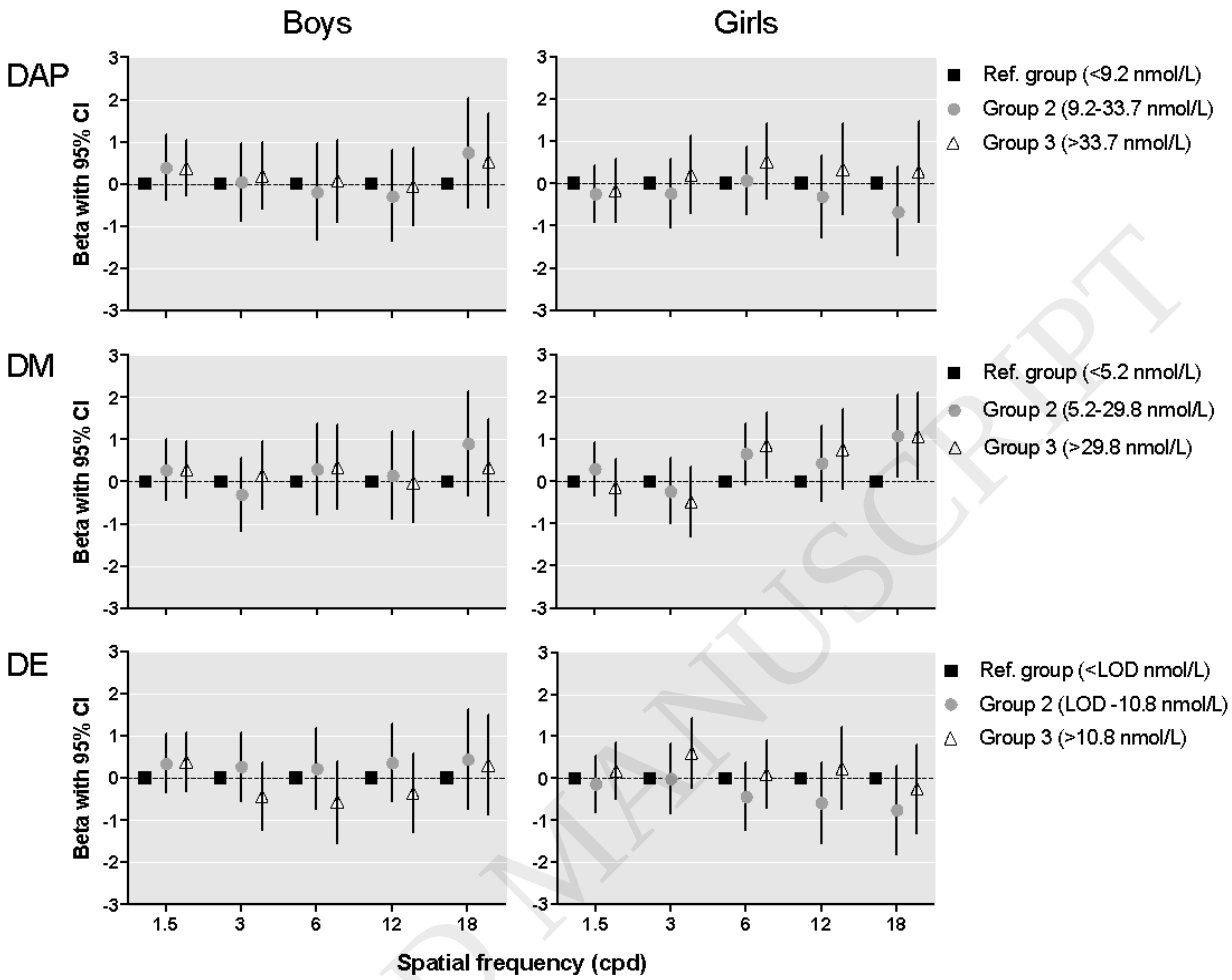

Figure 3. Associations between 6-years urinary metabolites and FACT scores.

Table 1. Descriptive characteristics of the participants

	All subjects (n=180)		
	N	%	Median (IQR)
Characteristics of mothers and families			
Mothers' age at study inclusion	180		30.4 (27.2-32.8)
Maternal educational level			
High school or less	62	34.4	
University level	118	65.6	
Smoking at inclusion (% yes)	180	23.3	
Alcohol comsumption at inclusion (% yes)1	180	12.8	
Fish intake (% ≥ 2 per week)	180	28.9	
Creatinine levels	180		980.5 (718-1345)
Mothers' IQ2	180		94 (84.5-101)
HOME score	180		46 (44-49)
Children's characteristics			
Gestational age (weeks)	180		40 (39-40)
Sex (% male)	180	51.1	
Parity			
0	77	42.4	
≥1	103	57.6	
Breastfeeding (%yes)	180	65	
Testing characteristics			
Lux	180		240 (100-665)
Child examiner (% psychologist 1)	180	49.4	
[1]Almost once a week			
2Measured with the Wechsler Adult Intelligence Scale			
SD: Standard deviation			
IQR: Interquartile range			

Table 2 Quantification frequency and molar concentrations of OP metabolites in urine samples (n=180)

		Quantification frequency							
		n	% ≥ LOQa		p10	p25	Median	p75	p90
Maternal urine samples									
DAP		165	91.67		8.96	19.26	47.98	86.65	141.61
DM		162	90		7.24	12.84	39.45	74.30	117.73
DE		89	49.44		0.36	2.26	13.34	31.51	50.57
		Detection frequency							
		n	% ≥ LODb		p10	p25	Median	p75	p90
Child's urine samples									
DAP		146	81.11		2.5	5.95	21.04	51.77	126.80
DM		115	63.88		2.14	4.11	15.23	43.73	121.92
DE		97	53.88		1.43	3.44	10.84	21.95	61.4
DAP: Dialkylphosphates; DM: Dimethylphosphate; DE: Diethylphsophate									
LOQ: proportion of urinary samples with a quantified for the metabolites of interest									
LOD: proportion of urinary samples with a detected value for the metabolites of interest									
aLOQ: 1.25, 1.7, .02, .2, 1, and .45 µg/L for DEP, DETP, DEDTP, DMP, DMTP and DMDTP, respectively									
bLOD: .2, .1, .005, .06, .32, and .13 µg/L for DEP, DETP, DEDTP, DMP, DMTP and DMDTP, respectively									
SD: Standard Deviation									

Table 3 Descriptive characteristics of FACT scores

	Among all children (n=180)		Among Girls (n=88)		Among Boys (n=92)		
	Mean	SD	Mean	SD	Mean	SD	p value
1.5 cpd (row A)	6.3	1.3	6.37	1.25	6.25	1.31	0.51
3 cpd (row B)	6.5	1.5	6.34	1.54	6.58	1.56	0.31
6 cpd (row C)	5.7	1.7	5.6	1.57	5.76	1.86	0.54
12 cpd (row D)	4	1.8	3.98	1.77	4.03	1.83	0.84
18 cpd (row E)	2.5	2.2	2.16	1.99	2.84	2.33	0.04*
Higher values indicate better performance							
cpd: cycles per degree of visual angle							
SD: Standard Deviation							
* $p < 0.05$							