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Prenatal organophosphate insecticide exposure and infant sensory function

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Abstract

Background—Occupational studies suggest that exposure to organophosphate insecticides (OPs) can lead to vision or hearing loss. Yet the effects of early-life exposure on visual and auditory function are unknown. Here we examined the effects of prenatal OP exposure on grating visual acuity (VA) and auditory brainstem response (ABR) during infancy.

Methods—30 OPs were measured in umbilical cord blood using gas chromatography tandem mass spectrometry in a cohort of Chinese infants. Grating visual acuity (VA) (n=179–200) and auditory brainstem response (ABR) (n=139–183) were assessed at 6 weeks, 9 months, and 18 months. Outcomes included VA score, ABR wave V latency and central conduction time, and head circumference (HC). Associations between sensory outcomes during infancy and cord OPs were examined using linear mixed models.

Results—Prenatal chlorpyrifos exposure was associated with lower 9-month grating VA scores; scores were 0.64 (95% CI: −1.22, −0.06) points lower for exposed versus unexposed infants (p=0.03). The OPs examined were not associated with infant ABR latencies, but chlorpyrifos and phorate were both significantly inversely associated with HC at 9 months; HCs were 0.41 (95% CI: 0.75, 0.6) cm and 0.44 (95% CI: 0.88, 0.1) cm smaller for chlorpyrifos (p=0.02) and phorate (p=0.04), respectively.

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Conclusions—We found deficits in grating VA and HC in 9-month-old infants with prenatal exposure to chlorpyrifos. The clinical significance of these small but statistically significant deficits is unclear. However, the disruption of visual or auditory pathway maturation in infancy could potentially negatively affect downstream cognitive development.

Keywords

Pesticide; Chlorpyrifos; Auditory processing; Visual acuity; Neurodevelopment

Introduction

Synthetic pesticides are employed for pest management in a wide variety of agricultural, residential, occupational, and industrial settings worldwide. The largest consumer of pesticides is by far the agricultural sector. Annual global estimates report that nearly five million tons of pesticides are applied to crops each year (U.S.EPA, 2011; Zhang et al., 2011). China is one of the largest consumers of pesticides (Ding and Bao, 2013; U.S.EPA, 2011; Zhang et al., 2011). Synthetic pesticide use in Chinese agriculture is reported to be up to five times the global average per field unit (Zhang et al., 2014). Agricultural pesticide applications are thought to be even higher in Zhejiang province, the site of this study, at nearly twice the national rate (Huang et al., 2001).

Organophosphate insecticides (OPs) are the most heavily used class of pesticides in China's agricultural sector (Ding and Bao, 2013) and account for more than one-third of overall insecticide use there (Zhang et al., 2014). The primary route of OP exposure in China is thought to be via consumption of food grown in OP-treated fields. Chinese national food surveys have found that over 10% of fruits, vegetables, and cereal grains contain OP residues higher than the national safety standards and OPs, such as methamidophos, that have been banned for years are still regularly detected (Chen et al., 2012; Wang et al., 2008; Wang et al., 2013). Additional OP exposure may also occur from the consumption of contaminated drinking water or dust, topical treatments, residential pest control applications for common household pests (e.g., termites, cockroaches), or aerial spraying for mosquitoes (Bai et al., 2013; Huang et al., 2001; NPIC, 2010; U.S.CDC, 2016).

The mechanism of acute OP neurotoxicity is the inhibition of acetylcholinesterase (AChE). This leaves the neurotransmitter acetylcholine unchecked and results in the hyperstimulation of cholinergic receptors in the central nervous system (Kamanyire and Karalliedde, 2004). Cholinergic toxicity following acute or high OP exposures has been associated with a variety of deficits in neurological function in both laboratory animals and occupationally exposed adults (Abdollahi and Karami-Mohajeri, 2012; Kamanyire and Karalliedde, 2004; Yang and Deng, 2007).

OPs have also emerged as a concern for developmental neurotoxicity, even at relatively low levels of exposure where cholinergic toxicity would not be present. Due to concerns of early-life neurotoxicity, a number of commonly used OPs have been banned for residential uses in the U.S., China, and European Union (U.S.EPA, 2011; Zhang et al., 2011). Rapidly developing fetal brains may be susceptible to possible long-term effects of prenatal OP exposure (Garcia et al., 2005). Studies of prenatal exposure to OPs provide evidence of

associations with neurological effects in childhood, such as IQ deficits (Bouchard et al., 2011; Engel et al., 2011; Rauh et al., 2011) and cognitive delays (Bouchard et al., 2011; Engel et al., 2011; Eskenazi et al., 2007; Rauh et al., 2011; Rauh et al., 2006), as well as increased diagnoses of autism (Shelton et al., 2014), attention deficit-hyperactivity (Marks et al., 2010; Rauh et al., 2006), and pervasive developmental (Eskenazi et al., 2007; Rauh et al., 2006) disorders.

Despite a growing body of evidence regarding early-life OP exposure and these commonly studied neurodevelopmental and cognitive endpoints, much less is known about how exposure to OPs may affect childhood sensory functions, such as visual and auditory function. Proper visual and auditory system development in infancy is crucial to later learning processes, such as the development of language and other forms of communication, as well providing the foundation for reading skills in childhood (Algarin et al., 2003; Chonchaiya et al., 2013). Only two epidemiological studies to date have examined prenatal OP exposure and either visual or auditory-related outcomes (Handal et al., 2008; Sturza et al., 2016). Maternal self-reported occupational OP exposure during pregnancy was associated with significantly higher odds of poor visual acuity in infants (Handal et al., 2008), while number of pesticides (OPs and other classes) in cord blood was associated with slower auditory signal transmission in infants (Sturza et al., 2016). These studies provide some preliminary evidence that prenatal OP exposure may be associated with deficits in early-childhood sensory-related functions, but are limited by imprecise exposure assessments.

The current study sought to investigate the extent to which prenatal OP exposure, as measured directly in cord blood, is associated with visual and auditory function at three time points in infancy.

Methods

Ethics Statement

Study protocols received institutional review board approval from both the University of Michigan and Zhejiang University Children's Hospital. Signed, informed consent was obtained from parents prior to study participation.

Study Sample

Pregnant women were recruited late in gestation (37–42 weeks) from Fuyang Maternal and Children's Hospital between 2008 and 2011. Fuyang is a largely rural county within Zhejiang province. Approximately two-thirds of the study population lived in a rural area, yet very few (4%) had a parent who worked in agriculture (Silver et al., 2016b). 359 women with healthy, uncomplicated, singleton pregnancies were enrolled into a longitudinal study of iron deficiency and infant neurodevelopment. 237 of their infants had a sufficient volume of cord blood for pesticide analysis. Infant development was assessed at three follow-up visits around 6 weeks, 9 months, and 18 months of age. Further description of this study population has been previously published (Silver et al., 2016b).

Organophosphate Insecticides (OPs)

Following delivery, 10 mL of cord blood was collected in a lavender EDTA tube and immediately frozen. Frozen blood samples were transferred twice weekly on dry ice from Fuyang to Hangzhou, where they were separated and stored at 80 C at Zhejiang University Children's Hospital. Blood samples were later transferred still frozen on dry ice to the Institute of Toxicology at Nanjing Medical University for further analysis. Plasma samples were analyzed for 24 OPs and 6 OP metabolites using gas chromatography tandem mass spectrometry (GC-MS/MS) (Perez et al., 2010; ThermoScientific). A detailed protocol for the determination of pesticides in cord blood has been described elsewhere (Silver et al., 2016b). Briefly, 800μL plasma samples were mixed with an equivalent amount of saturated ammonium sulfate, centrifuged, and supernatants were subjected to solid phase extraction for cleaning and pre-concentration. Analytes were eluted in dichloromethane and n-hexane and then eluates were concentrated and reconstituted in 10 μL toluene for analysis. OPs were separated using a TRACE GC Ultra gas chromatograph (Thermo Scientific) equipped with a TR-PESTICIDE II column and measured with a triple quadropole TSQ XLA mass spectrometer (Thermo Scientific). Limits of detection (LODs) were determined by analyzing fortified serum on a signal-to-noise ratio of three. Quality control samples were generated using plasma samples and a known amount of OP standard (0.675 or 1.35 ng/mL). Quality control samples and blanks were analyzed concurrently with samples.

Naled (100% detected) was treated as a continuous variable and log-transformed prior analysis to account for its right-skewed distribution. Methamidophos (63.3% detected) and trichlorfon (51.0% detected) were converted to 3-level ordinal variables (<LOD, medium, high [median split for those above LOD]; cut-offs were <1.5, 1.5–18.2, >18.2 ng/mL and <0.4, 0.4–1.7, >1.7 ng/mL, for methamidophos and trichlorfon, respectively). Chlorpyrifos (34.6% detected) and phorate (17.9% detected) were treated as dichotomous (<LOD/detect; cut-offs were <0.4, ≥0.4 ng/mL and <1.8, ≥1.8 ng/mL, for chlorpyrifos and phorate, respectively). A "number of OP detects" variable was created by assigning OPs <LOD a value of 0 and detects a value of 1; these were then summed to create an index of OP exposure for each infant (Wickerham et al., 2012). "Number of OP detects" was also treated as a continuous variable.

Grating Visual Acuity (VA)

Grating VA was estimated here using the Teller acuity card (TAC) preferential looking procedure. This test provides a quantitative measure of binocular grating acuity for infants and nonverbal children. Grating VA improves throughout in infancy and childhood with the maturation of the visual pathway in the developing brain (Tau and Peterson, 2010).

Grating VA was measured at three time points, 6 weeks, 9 months, and 18 months using a TAC procedure. The ambient lighting luminance was 85 candelas/m^2. Examiners were blinded to infant exposure status. Infants faced a TAC test stage (38 cm away) and were held upright by study staff. Examiners presented a series of mounted prints, with black and white vertical gratings to one side and a gray blank on the other side, through a rectangular opening in the test stage. Gratings ranged from coarse to fine (0.44–27 cycles/degree) and cards had 35% reflectance. Cards were presented in descending order, with wider (coarse)

gratings presented first. Gratings were presented on both the left and right sides of the print to avoid habituation. Examiners observed infant looking behavior through a small central aperture in the test stage and determined which card the infant looked at. Examiners repeated the presentation several times until a confident judgment could be made based on consistent looking toward the location of the grating. Grating VA score was estimated as the spatial frequency of the finest grating that the infant could resolve. If the tester was uncertain about the acuity estimate, a second examiner (blinded to the results of the first testing) re-tested the infant. If the infant was uncooperative, parents were asked to return for testing another day. Grating VA data was available for 196 infants at 6-week testing, 200 infants at 9-month testing, and 179 infants at 18-month testing.

Auditory Brainstem Response (ABR)

ABR measures electrical activity in the brain by quantifying the activation of neurons along the auditory pathway following an auditory stimulus. ABRs in infants consist of three prominent peaks or waves. Wave I corresponds to the activation of the distal cochlear nerve, wave III, the distal cochlear nuclei, and wave V, the lateral lemniscus nucleus (DeBonis and Donohue, 2008; Hall, 2007). Observed decreases in ABR peak latencies during infancy (i.e., increased rates of signal transmission) directly correspond to increasing maturation of the auditory pathways in the developing brain (Hecox and Galambos, 1974; Jiang, 1995).

ABR was measured in 6-week-, 9-month-, and 18-month-old infants during unsedated sleep using a Biologic Navigator (Bio-Logic Systems Corp., Mundelein, IL)/Traveler evoked potential system. Infants first underwent a standard hearing screening protocol. Stimuli for the hearing screening test were a series of square wave rarefaction monophasic clicks delivered to both ears by insert transducers with a presentation rate of 31.3/second, a duration of 100 μs, and an intensity of 30 dB, nHL. Infants who passed the hearing screening continued on to the ABR protocol. Stimuli for the ABR test were also square wave rarefaction monophasic clicks delivered to each ear by insert transducers with a presentation rate of 11.7/second, a duration of 100 μs, and an intensity of 80 dB, nHL. The recording epoch was 74.67 ms. ABRs were recorded by silver/silver chloride electrodes attached to infant's forehead in three locations: midline below the hairline (non-inverting), mastoid on ipsilateral mastoid (inverting) and contralateral mastoid (ground). The impedance was <10 kΩ. The program rejected ABR traces contaminated by high-amplitude artifacts (voltage > ±23.80 μV). 1300 sweeps were averaged for each run, and two succesive averages were obtained for each ear (2600 sweeps). Right and left ears were averaged (5200 sweeps) to obtain a single measurement for each infant.

ABR waveforms were analyzed for latencies for peaks I, III, and V. We focused on wave V latency and central conduction time (CCT), which is the inter-peak latency from wave V to wave I. These two measures are commonly used to gauge auditory processing because they are easy to identify and easily reproducible (Berglund et al., 2011). Wave V has long been used as a measure of the neurological integrity of the auditory system (Hecox and Galambos, 1974). Wave V ABR latencies were available for 183 infants at 6-week testing, 176 infants at 9-month testing, and 139 infants at 18-month testing. Other ABR data (waves

I and III) were available for 182, 154, and 106 infants for the 6-week, 9-month and 18-month time points, respectively.

Covariates

Sex was recorded at the time of birth. Cord blood iron status was defined based on serum ferritin, which was measured by chemiluminescent immunoassay (IMMULITE, Diagnostic Products) and categorized as iron deficient or sufficient (≤75 and >75 μg/L). Serum ferritin values >370 μg/L were excluded due to the possibility of infection or inflammation. Infant age was recorded at the 6-week, 9-month, and 18-month testings. Maternal education, occupation, and family income were obtained by maternal self-report from a family background questionnaire administered at the 6-week follow-up visit. Season of testing was defined by categorizing the month of the developmental testing into spring (March-May), summer (June-September), or fall/winter (October-February). Head circumference was measured twice and the average of the two measurements was taken at the 6-week, 9-month, and 18-month follow-up visits using a soft plastic tape placed just above the eyebrows and wrapped around the widest part of the head.

Statistical analysis

Statistical analyses were conducted using SAS 9.3 (Cary, North Carolina). Descriptive statistics and frequencies were examined for all variables of interest, including sex, age at sensory testing, cord ferritin, gestational age, birth weight, maternal education and occupation, family income, and season of testing. To explore the possibility of retention bias, levels of OP exposure for those with and without sensory data were compared across the three time points.

Linear mixed models (LMM) with random intercepts were used to evaluate associations between cord OP exposures and either grating VA scores or ABR outcomes (wave V latency, CCT) at 6 weeks, 9 months, and 18 months. Given our relatively small sample sizes, especially for the 18-month time point, we took a conservative approach to choosing covariates for our models. Sex, age at testing, and cord ferritin were chosen *a priori*. Additional covariates considered for inclusion were maternal education and occupation, income, and season in which neurological testing took place. Bivariate analyses revealed that season of testing was the only variable with the potential to be a true confounder, since it was associated with both OP exposure and the outcomes. However, inclusion of season in the models did not significantly influence the results. Therefore, to maximize power, we chose the most parsimonious models, adjusting for sex, age at testing, and cord ferritin. Initial LMM results indicated very small differences overall in longitudinal outcomes between exposure groups. Therefore, to enable more meaningful comparisons of effect estimates at each of the three time points, we used a least squared means (lsmeans) approach and included “time” as a class variable and “time*OP” in our LMM models. For continuous exposures (number of OP detects, log-naled), the parameter of interest was the slope estimating change in 6-week, 9-month or 18-month VA score or ABR latencies per 1 unit increase in OP. For categorical exposures (methamidophos, trichlorfon, chlorpyrifos, phorate), the parameter of interest was the difference in mean 6-week, 9-month or 18-month VA score or ABR latencies by category of OP exposure.

To identify sex dimorphic effects, we ran the LMM models stratified by infant sex. We completed two additional sensitivity analyses to further understand the effect of iron status on associations between OPs and sensory outcomes. First, we examined the potential effects of iron deficiency as an effect modifier by stratifying by cord iron status (sufficient/deficient). Iron deficiency was previously found to exacerbate the effect of overall pesticide exposures on ABR latencies (Sturza et al., 2016). We additionally re-ran our models using a longitudinal iron status variable (cord and 9 month [18 month was unavailable]) to determine if iron status later in infancy impacted our results at 9 or 18 months.

We further considered the possibility that prenatal exposure might be associated with reduced head circumference (Berkowitz et al., 2004). Head circumference may be directly associated with auditory pathway length and has been used as a rough proxy for pathway length in previous studies of lead exposure and ABR (Rothenberg et al., 2000; Silver et al., 2016a). Therefore, we explored associations between prenatal OP exposure and head circumference at our three time points, as well as head circumference as a confounder of our OP/ABR analyses.

Results

Of the 30 OPs or metabolites measured, five were detected in ≥10% of cord blood samples: naled, methamidophos, trichlorfon, chlorpyrifos, and phorate. Distributions of these detectable OPs in the cord blood of our study sample are shown in Table 1. There were no underlying differences in OP exposure among those with and without sensory data at 6 weeks or 9 months (Table S1). Many infants were missing ABR wave I and III data at 18 months (n=132); methamidophos exposure was significantly different among infants with and without 18-month ABR data (Table S1). There were no significant differences in cord blood pesticides by infant sex (Table S2). Grating VA was not correlated across time points; Spearman correlations were 0.02 (p=0.77), 0.003 (p=0.97), and 0.04 (p=0.58) for VA at 6 weeks/9 months, 6 weeks/18 months and 9 months/18 months, respectively. ABR outcomes were highly correlated across time points with ρ's ranging from 0.68 to 0.96 (p<0.0001) for wave V and CCT outcomes.

Sample characteristics are shown in Table 2. An average of 3.0 (±1.6 SD) OPs were detected in cord blood from each subject. Infants all had birth weights ≥2.5 kg and were carried to term (37–42 weeks). Further characteristics of the study population have been reported previously (Silver et al., 2016b).

Grating VA

Adjusted LMM results for grating VA score are shown in Table 3. In general, concentrations of OPs in cord blood were associated with lower grating VA scores at 9 months, though this association was only statistically significant for chlorpyrifos. Infants with prenatal exposure to chlorpyrifos had 9-month grating VA scores that were, on average, 0.64 points lower than unexposed infants (p=0.03). Prenatal OP exposure was not significantly associated with VA scores at 6 weeks or 18 months (Table 3).

ABR

Adjusted LMM results for the ABR outcomes are shown in Table 4. Prenatal OP exposure was not significantly associated with auditory signal transmission speed at any of the three time points (Table 4).

Sex-stratified grating VA

Bivariate analyses revealed that grating 6-week VA scores differed significantly by infant sex (Table S3). 6-week scores were lower in males compared to females; means (SD) were 1.07 (0.37) for boys and 1.28 (0.68) for girls (p=0.01). VA scores did not significantly differ by sex at the other time points (Table S3). Sex-stratified LMM results for grating VA are shown in Figure 1. There were no noticeable differences by sex at the early time points. However, by 18 months, sex-differences emerged for some of the exposures measured (OP detects, methamidophos, chlopryrifos and phorate). For these four exposures, VA scores seem to be consistently lower in exposed girls and consistently higher in exposed boys. For example, 18-month VA scores were 0.94 points lower for chlorpyrifos-exposed girls, compared to unexposed (p=0.08), while scores were 0.68 points higher for exposed boys, compared to unexposed (p=0.08) (Figure 1). For girls, the effect of prenatal exposure to overall number of OP detects and chlorpyrifos seems to become stronger (more negative) with increasing age, though tests for trend were not significant; p=0.55 and p=0.45, for OP detects and chlorpyrifos, respectively.

Sex-stratified ABR

Bivariate analyses revealed that ABR scores differed significantly by infant sex at all three time points (Table S4). Wave V and CCT latencies were consistently higher in girls compared to boys. For example, at 6 weeks, Wave V means (SD) were 6.45 (0.26) ms for boys and 6.55 (0.27) ms for girls (p<0.0001). Similar effects were seen for the other time points (Table S4). Despite this, sex-stratified LMM results for ABR were inconclusive, as shown in Figure 2 (6 weeks) and Table S5 (9 and 18 months). The only consistent sex-specific difference across ABR outcomes was observed for naled at 6-weeks. Estimates were -0.011 and -0.003 for boys, for wave V and CCT, respectively, while for girls they were 0.017 and 0.030, respectively (Figure 2). These small differences were not statistically significant.

Iron status

Further analysis of our ABR models stratified by cord iron status (sufficient/deficient) did not show evidence of effect modification by iron deficiency in our sample (Table S6). Inclusion of longitudinal iron status in our models did not significantly alter the findings but slightly strengthened the association between chlorpryifos and 9-month grating VA; β (95% CI)= -0.81 (-1.41, −0.22).

Head circumference

Our exploration of head circumference revealed that infants prenatally exposed to chlorpyrifos and phorate had reduced head circumferences at 9 months, compared to unexposed infants (Table 5). Head circumferences were 0.41 (95% CI: 0.75, 0.6) cm and

0.44 (95% CI: 0.88, 0.1) cm smaller in infants exposed to chlorpyrifos (p=0.02) and phorate (p=0.04), respectively, compared to unexposed. However, despite evidence of these associations, when we included head circumference in our OP/ABR models, we did not find any evidence of confounding (results not shown).

Discussion

Here we found that infants prenatally exposed to chlorpyrifos had lower grating VA scores at 9 months, compared to infants with exposures <LOD. Other individual OP exposures were not associated with VA scores at 6 weeks, 9 months, or 18 months. By 18 months of age, VA scores were consistently lower in girls with more prenatal exposure to overall OPs, methamidophos, chlorpyrifos, and phorate, compared to those with exposures <LOD, while scores were consistently higher in exposed boys, compared to those with exposures <LOD. Prenatal OP exposure was not significantly associated with infant ABR latencies in our cohort. Sex-specific analyses of associations between prenatal OPs and infant ABRs were also inconclusive. We did not see any effect modification by cord iron status nor did we find any confounding by infant head circumference, despite observing reduced head circumferences in infants exposed to chlorpyrifos and phorate prenatally.

To our knowledge there are only two previous studies that have examined the effects of prenatal OP exposure on visual- or auditory-related functions in infancy or childhood. Ecuadorian infants, aged 3–23 months, whose mothers were exposed to unspecified OPs during pregnancy through their work in the cut-flower industry, had nearly five times higher odds of poor visual acuity, compared to infants whose mothers did not work in the industry (Handal et al., 2008). We similarly found deficits in visual acuity at 9 months in infants prenatally exposed to chlorpyrifos. We did not see deficits in VA at 6 weeks or 18 months however. It may be the case that 6 weeks is too early to observe significant effects on these outcomes. Assessment of infants at this very young age is likely to be prone to more error, especially for this outcome. Similarly, the smaller sample size at 18 months may have limited our power to detect an effect. VA scores were also not correlated across time points, which also may explain some of the variation in the results.

Our previous small pilot study of 9-month-old Chinese infants found that number of pesticides (mixed classes, including OPs) detected in cord blood was positively associated with ABR wave V latencies and CCTs (Sturza et al., 2016). The association with CCT was additionally modified by iron status, such that effect estimates were larger in the low cord ferritin group. The pilot study did not find any significant associations between number of OP detects and ABR latencies, and no individual OPs were examined due to detection rates <50% (Sturza et al., 2016). In the present study, we similarly did not find any associations between number of OP detects or individual OPs and ABR latencies, but, contrary to the early pilot results, did not see any effect modification by iron status.

Other studies have previously examined prenatal exposure to OPs and infant head circumference with mixed results. Several studies found increases in head circumference (Eskenazi et al., 2004; Harley et al., 2011) or no association (Perera et al., 2003; Whyatt et al., 2004) with maternal OP exposure during pregnancy. While others reported inverse

associations between head circumference at birth and maternal levels of chlorpyrifos (Berkowitz et al., 2004) and OP metabolites, diethylphosphate (DEP) (Huang et al., 2017), diethylalkylphosphates (DEAPs) and dialkylphosphates (DAPs) (Naksen et al., 2015). Two studies found that the effect of prenatal OPs on head circumference was modified by paraoxonase (PON1) phentoype (Berkowitz et al., 2004; Harley et al., 2011). While we did not examine PON1, we did find significant deficits in 9-month head circumference following prenatal chlorpyrifos exposure. It is unclear why we observed significant effects on head circumference at 9 months only and not the earlier or later time point. Infants are rapidly developing and trajectories of head circumference growth are not linear (WHO, 2017). It may be the case that the timing of head circumference measurements is contributing to the seemingly transient effects seen here for chlorpyrifos and phorate. There may also be concurrent environmental and/or nutritional factors that could potentially affect head circumference that were not measured in this study.

Occupational studies and case studies of OP exposure provide evidence of adverse ocular and auditory effects in adults. Vision loss (Pham et al., 2016), retinopathy (Pham et al., 2016), myopia (Dementi, 1994), Saku disease (Dementi, 1994), and retinal (Dementi, 1994) and macular degeneration (Misra et al., 1985) have all been reported in rural workers exposed to high levels of OPs. Auditory-related abnormalities, such as hearing loss (Hoshino et al., 2008), deficits in auditory temporal processing (Camarinha et al., 2011), and delays in auditory stimulation classification (Dassanayake et al., 2008), have also been found in OP-exposed workers.

The mechanism of acute toxicity elicited by high exposures to OPs is well understood. OPs inhibit acetylcholinesterase (AChE), the enzyme responsible for terminating the neurotransmitter acetylcholine’s activity. Without functional AChE, acetylcholine builds up in the synapse, leading to hyperstimulation of the cholinergic receptors at neuronal and neuromuscular junctions (Abdollahi and Karami-Mohajeri, 2012; Eddleston et al., 2008; Kamanyire and Karalliedde, 2004). However, low-dose exposure levels, typical in non-occupational settings, do not usually elicit cholinergic toxicity or acetylcholinesterase inhibition. Yet neurodevelopmental toxicity is still observed. The most well-studied OP, chlorpyrifos, has been demonstrated to disrupt neuronal processes such as neuron replication and differentiation, axon formation, synaptogenesis, apoptosis, and neural circuit formation, even at low doses where cholinergic toxicity is not present (Slotkin, 2004).

Changes in brain morphology following low-dose early-life chlorpyrifos exposure have also been reported in laboratory rats and human children. Chlorpyrifos in the early postnatal period in rodents has been shown to affect both numbers and types of glial cells and neurons in brain regions associated with cognition, mood, and behavior (Roy et al., 2004; Roy et al., 2005). Postnatal chlorpyrifos has also been associated with glial scarring, a common response to cellular injury (Roy et al., 2005), while prenatal chlorpryifos was associated with glial cell markers (Garcia et al., 2002). A recent study of school children similarly found that prenatal chlorpyrifos exposure was associated with enlargements of white matter in the superior temporal (MST), posterior middle temporal (MT), inferior postcentral gyri, frontal gyrus, gyrus rectus, cuneus, and precuneus of the brain (Rauh et al., 2012). The authors speculated that the increased white matter may be representative of glial scarring, similar to

the effects seen in rodents (Rauh et al., 2012; Roy et al., 2005). Both the MST and the MT are part of the extrastriate visual cortex (Blumberg and Kreiman, 2010) and are involved in processing visuospatial information (Born and Bradley, 2005; Maunsell, 1995). Lesions in the MT have been associated with visual deficits in monkeys (Born and Bradley, 2005). The cuneus is also thought to play a role in the signaling between the primary visual cortex and the extrastriate visual cortices (Vanni et al., 2001). It is unclear whether the MST, MT, or cuneus might also be related to grating acuity in infancy. Given that prenatal chlorpyrifos has been associated with increased white matter or glial scarring in the MST or MT in children, it is possible that this may be one pathway that chlorpyrifos could affect visual function.

Our study is limited in several ways. OPs are non-persistent with short half-lives. Thus, having measures of exposure only at birth limited our ability to address the temporal variability of OP exposure during pregnancy and infancy. Due to this shortcoming, we were unable to characterize exposure at all sensitive developmental stages (Eskenazi et al., 2007). Since this was part of a larger study of 96 pesticides and metabolites, our methods were not optimized for OP detection (Silver et al., 2016b), likely resulting in higher detection limits and great numbers of non-detects, compared to a more targeted approach. OP levels in blood tend to be low anyway, likely also contributing to the high levels of non-detects (Barr et al., 1999). The many non-detects necessitated the use of crude exposure categories (<LOD/detect or <LOD/medium/high) for nearly all of the OPs examined, thereby limiting the scope of our statistical analyses. We also performed many statistical analyses and it is therefore possible that some of our findings may be attributable to chance. Additionally, although our neurodevelopmental testers were highly trained, assessing young infants, as we did here, augments the chance of error, particularly for grating VA. Furthermore, solely presenting the VA gratings in descending order, as is recommended in the testing manual, may result in habituation, which could possibly confound the estimated VA score. This issue can be addressed by presenting the cards in both descending and ascending order. However, the limited attention span of young infants necessitated a quick completion of the testing, thereby eliminating this as a viable option. Finally, the findings from our relatively small cohort may not be generalizable to infants in other parts of the world, especially considering that all the infants included in this study were carried to term and otherwise healthy. Low birth weight or pre-term infants may be more likely to have delayed development. The effects of prenatal OPs on infant sensory function in these vulnerable populations should be assessed in future work.

Despite its limitations, this study has a number of strengths. It used specific measurements of OP parent compounds in umbilical cord blood to assign prenatal exposure, thus providing direct evidence of fetal exposure (Barr et al., 1999; Munoz-Quezada et al., 2013), and augmenting the utility of the findings for regulatory considerations. Additionally, OP levels in cord blood are likely to reflect the available dose, since the OPs have not yet been eliminated from the infant's body (Needham et al., 1995). Of the previously published studies of prenatal OPs and visual or auditory function, one used a crude "number of pesticide detects" in cord blood to define exposure (Sturza et al., 2016), while the other used self-reported maternal occupational exposure during pregnancy (Handal et al., 2008). The current study also examined a large number of OPs (18 detected out of 30 analyzed), many

of which have not been previously examined for neurodevelopmental effects in humans. Additionally, we assessed sensory development at three time points (6 weeks, 9 months, and 18 months). The longitudinal design gives a more comprehensive view of overall sensory development in infancy than previous studies. The tests of sensory function, ABR and grating VA, provided a non-invasive way of measuring auditory and visual function and maturation throughout infancy.

Conclusions

Prenatal exposure to chlorpyrifos was significantly associated with deficits in grating visual acuity at 9 months. Chlorpyrifos and phorate were also both associated with deficits in head circumference at 9 months. These effects were not observed at 6 weeks or 18 months. The clinical significance of these statistically significant, yet seemingly transient, deficits are unclear, yet warrant further study given that chlorpyrifos and phorate are used worldwide. The proper maturation of the visual and auditory pathways in infancy provides the foundation for later learning processes in childhood. Disruption of this essential neurodevelopmental stage could potentially have detrimental effects on downstream cognition and other developmental processes.

Acknowledgments

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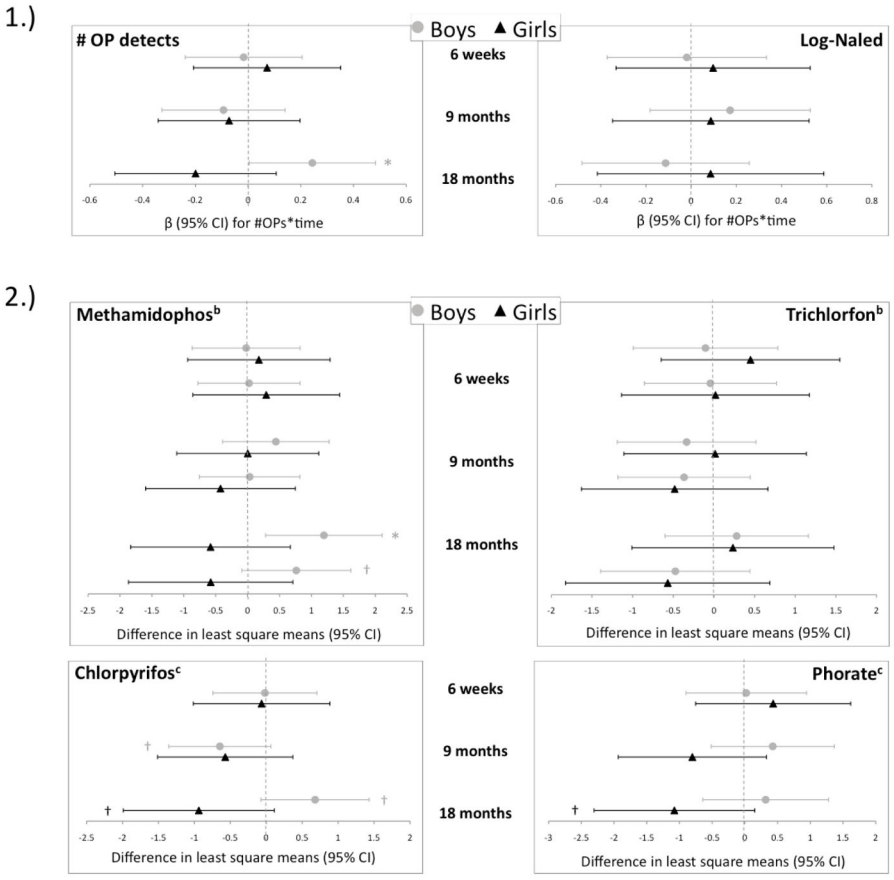

Figure 1.

Sex-stratified change/difference (95%) in grating VA scores over infancy by prenatal OP exposure [a]

1.) Estimated change in grating VA score per 1 unit increase in OP exposure

2.) Difference in mean grating VA score by category of OP exposure

[a]Models adjusted for age at testing and cord ferritin

[b]Categories of OP exposure: high versus ND and medium versus ND

[c]Categories of OP exposure: exposed versus ND

High/Medium/ND cut-offs (ng/mL): methamidophos >18.2/1.5–18.2/ND; trichlorfon >1.7/0.4–1.7/ND; chlorpyrifos ≥0.04/ND; phorate ≥1.8/ND

†p<0.10, *p<0.05, **p<0.01

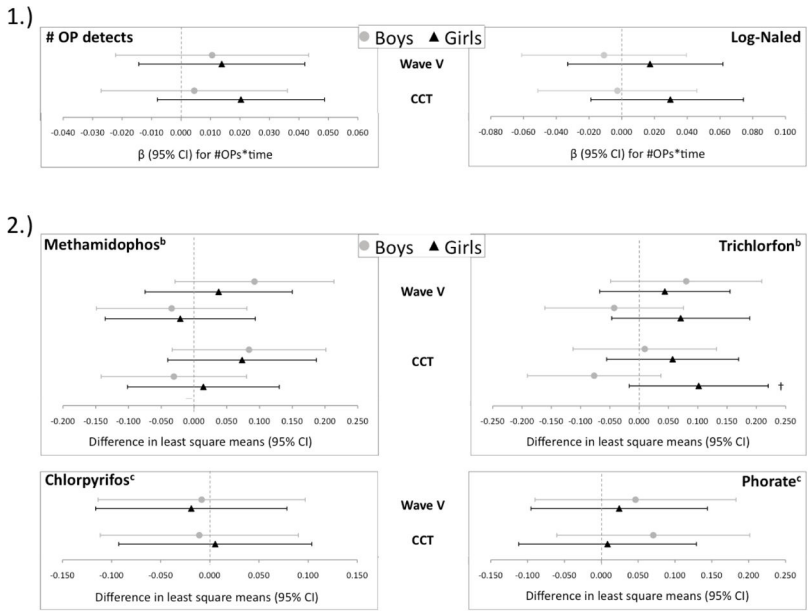

Figure 2.

Sex-stratified change/difference (95%) in 6-week ABR scores by prenatal OP exposure [a]

1.) Estimated change in ABR latency per 1 unit increase in OP exposure

2.) Difference in mean ABR latency by category of OP exposure

[a]Models adjusted for age at testing and cord ferritin

[b]Categories of OP exposure: high versus ND and medium versus ND

[c]Categories of OP exposure: exposed versus ND

High/Medium/ND cut-offs (ng/mL): methamidophos >18.2/1.5–18.2/ND; trichlorfon >1.7/0.4–1.7/ND; chlorpyrifos ≥0.04/ND; phorate ≥1.8/ND

†p<0.10, *p<0.05, **p<0.01

Table 1

Distributions of OP concentrations (ng/mL) detected in at least 10% of umbilical cord blood plasma samples from infants with 6-week sensory data (n=196)

OP Exposure	LOD	#> LOD (%)	Min.	25th	50th	75th	90th	95th	Max.
Chlorpyrifos	0.40	68 (34.6)	ND	ND	ND	0.50	1.80	2.57	7.33
Methamidophos	1.52	124 (63.3)	ND	ND	5.03	26.00	63.85	116.15	280.18
Naled	0.42	196 (100)	0.50	0.92	1.56	4.58	9.58	15.54	29.23
Phorate	1.79	35 (17.9)	ND	ND	ND	ND	3.37	5.32	50.13
Trichlorfon	0.35	100 (51.0)	ND	ND	0.45	1.68	3.74	5.66	29.14
# OP detects	NA	NA	1.0	2.0	3.0	4.0	5.0	6.0	8.0

OPs detected in <10% of samples (LOD [ng/mL], % detection): acephate (0.10, 2.6%); chlorpyrifos-methyl (0.01, 6.0%); diazinon (0.003, 0.5%); isofenphos-methyl (0.13, 1.0%); omethoate (1.35, 9.1%); mevinphos (0.12, 4.5%); terbufos (0.33, 1.0%); carbophenothion sulfone (0.02, 6.1%); DEDTP (0.06, 8.7%); DMDTP (1.74, 3.1%); phorate sulfone (0.01, 2.0%); TCPY (2.32; 1.0%)

OPs not detected (LOD [ng/mL]): dichlorvos (0.01); dicrotophos (0.003); dimethoate (0.01); dimethylvinphos (0.003); fensulfothion (0.03); formothion (0.01); fosthiazate (0.07); malathion (0.002); methidathion (0.07); methyl-parathion (0.04); monocrotophos (0.01); DMTP (1.35)

Table 2

Study sample characteristics for infants with available sensory data, Zhejiang province, China (n=200)

	6 weeks		9 months		18 months	
Variable	**N**	**Mean (SD)**	**N**	**Mean (SD)**	**N**	**Mean (SD)**
Outcome						
Grating VA score	196	1.17 (0.55)	200	7.65 (2.33)	179	9.46 (2.57)
ABR wave V	183	6.45 (0.26)	176	5.90 (0.26)	139	5.71 (0.23)
ABR CCT	182	4.94 (0.27)	153	4.40 (0.25)	106	4.20 (0.21)
Potential covariates						
Age at testing (days)	196	43.3 (5.1)	200	282.8 (10.4)	179	554.7 (10.6)
Head circumference (cm)	199	38.0 (1.2)	199	45.1 (1.5)	186	47.4 (1.4)
Birth weight (kg)*	200	3.4 (0.4)				
Gestational age (wks)*	200	39.6 (1.0)				
	N	**N (%)**				
Sex	200					
Male		107 (53.5)				
Female		93 (46.5)				
Serum ferritin (μg/L)*	199					
Low (≤75)		39 (19.6)				
Normal (75–370)		160 (80.4)				
Maternal occupation	186					
Housewife		75 (40.3)				
Other		111 (59.7)				
Maternal education	186					
Middle school or less		71 (38.2)				
High/secondary school		53 (28.5)				
College		62 (33.3)				
Family income (Yuan/yr)	183					
<30,000		38 (20.1)				
30,000–49,999		35 (19.1)				

Variable	6 weeks		9 months		18 months	
	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)
50,000–999,999		58 (31.7)				
≥ 100,000		52 (28.4)				

* Measured at birth

Abbreviations: VA, grating visual acuity; ABR, auditory brainstem response; CCT, central conduction time

Table 3

Adjusted [a] longitudinal change/difference in VA score over infancy by prenatal OP exposure

	Grating VA score		
OP insecticide	**6 weeks(n=195)**	**9 months(n=198)**	**18 months(n=164)**
Continuous	**β (95% CI) for OP[b]**		
# OP Detects	0.03	−0.10	0.02
	(−0.15, 0.20)	(−0.28, 0.08)	(−0.17, 0.21)
Log-Naled	0.06	0.11	−0.01
	(−0.21, 0.34)	(−0.16, 0.39)	(−0.31, 0.29)
3-level (High/Med./ND)	**Difference in least square means (95% CI)[c]**		
Methamidophos	0.09	0.19	0.39
(High vs ND)	(−0.60, 0.78)	(−0.49, 0.87)	(−0.37, 1.14)
Methamidophos	0.16	−0.15	0.23
(Med. vs ND)	(−0.53, 0.84)	(−0.83, 0.53)	(−0.52, 0.97)
Trichlorfon	0.15	−0.22	0.21
(High vs ND)	(−0.54, 0.85)	(−0.91, 0.47)	(−0.52, 0.94)
Trichlorfon	−0.06	−0.46	−0.57
(Med. vs ND)	(−0.74, 0.62)	(−1.14, 0.21)	(−1.33, 0.18)
2-level (Detect/ND)	**Difference in least square means (95% CI)[c]**		
Chlorpyrifos	−0.04	−0.64 *	−0.05
(Detect vs ND)	(−0.63, 0.55)	(−1.22, −0.06)	(−0.68, 0.58)
Phorate	0.20	−0.28	−0.37
(Detect vs ND)	(−0.54, 0.94)	(−1.02, 0.45)	(−1.13, 0.40)

[a] LMM models adjusted for sex, age at testing, and cord ferritin

[b] Estimated change in VA score per 1 unit increase in OP

[c] Difference in mean VA score

High/Medium/ND cut-offs (ng/mL): methamidophos >18.2/1.5–18.2/ND; trichlorfon >1.7/ 0.4-1.7/ND; chlorpyrifos ≥0.04/ND; phorate ≥1.8/ND

Abbreviations: ND, non-detect (below limit of detection [<LOD]); VA, grating visual acuity

* p<0.05

Table 4

Adjusted [a] longitudinal change/difference in ABR latencies over infancy by prental OP exposure

	ABR outcome					
	6 weeks		9 months		18 months	
OP insecticide	**Wave V (n=181)**	**CCT (n=180)**	**Wave V (n=165)**	**CCT (n=146)**	**Wave V (n=126)**	**CCT (n=94)**
Continuous	**β (95% CI) for OP[b]**					
# OP Detects	0.01 (−0.01, 0.03)	0.01 (−0.01,0.03)	−0.02 (−0.04, 0.01)	−0.01 (−0.03, 0.01)	0.00 (−0.03, 0.02)	0.00 (−0.03, 0.02)
Log-Naled	0.00 (−0.03, 0.04)	0.01 (−0.02, 0.05)	0.00 (−0.04, 0.03)	0.01 (−0.02, 0.05)	0.00 (−0.04, 0.04)	0.00 (−0.04, 0.04)
3-level (High/Med./ND)	**Difference in least square means (95% CI)[c]**					
Methamidophos (High vs ND)	0.05 (−0.03, 0.14)	0.07† (−0.01, 0.15)	0.00 (−0.08, 0.09)	0.06 (−0.02, 0.15)	0.02 (−0.07, 0.11)	0.05 (−0.04, 0.15)
Methamidophos (Med. vs ND)	−0.02 (−0.11, 0.06)	−0.01 (−0.09, 0.07)	−0.02 (−0.10, 0.06)	−0.03 (−0.11, 0.06)	−0.03 (−0.12, 0.06)	0.01 (−0.09, 0.11)
Trichlorfon (High vs ND)	0.04 (−0.04, 0.13)	0.02 (−0.06, 0.11)	−0.02 (−0.10, 0.06)	−0.02 (−0.10, 0.07)	−0.02 (−0.11, 0.07)	0.01 (−0.08, 0.11)
Trichlorfon (Med. vs ND)	0.00 (−0.08, 0.08)	0.00 (−0.08, 0.08)	−0.04 (−0.13, 0.05)	−0.04 (−0.13, 0.04)	0.01 (−0.09, 0.10)	−0.02 (−0.11, 0.08)
2-level (Detect/ND)	**Difference in least square means (95% CI)[c]**					
Chlorpyrifos (Detect vs ND)	−0.02 (−0.09, 0.05)	−0.01 (−0.08, 0.06)	−0.02 (−0.10, 0.05)	−0.01 (−0.08, 0.06)	−0.01 (−0.09, 0.07)	−0.04 (−0.12, 0.04)
Phorate (Detect vs ND)	0.03 (−0.06, 0.11)	0.03 (−0.05, 0.12)	−0.06 (−0.15, 0.04)	−0.02 (−0.11, 0.07)	0.01 (−0.09, 0.10)	−0.01 (−0.11, 0.08)

[a] LMM models adjusted for sex, age at testing, and cord ferritin

[b] Estimated change in ABR latency per 1 unit increase in OP

[c] Difference in mean ABR latency

High/Medium/ND cut-offs (ng/mL): methamidophos >18.2/1.5–18.2/ND; trichlorfon >1.7/ 0.4–1.7/ND; chlorpyrifos ≥0.04/ND; phorate ≥1.8/ND Abbreviations: ND, non-detect (below limit of detection [<LOD]); ABR, auditory brainstem response

† p<0.10,

* p<0.05

Table 5

Adjusted [a] longitudinal change/difference in head circumference over infancy by prenatal OP exposure

	Head circumference (cm)		
OP insecticide	**6 weeks (n=199)**	**9 months (n=199)**	**18 months (n=186)**
Continuous	**β (95% CI) for OP[b]**		
# OP Detects	0.04	−0.07	−0.02
	(−0.06, 0.14)	(−0.17,0.04)	(−0.12, 0.09)
Log-Naled	0.09	0.02	0.07
	(−0.07, 0.25)	(−0.14, 0.19)	(−0.10, 0.23)
3-level (High/Med./ND)	**Difference in least square means (95% CI)[c]**		
Methamidophos	0.28	0.20	0.25
(High vs ND)	(−0.12, 0.68)	(−0.21, 0.60)	(−0.17, 0.67)
Methamidophos	0.39 †	0.35	0.42 †
(Med. vs ND)	(−0.00, 0.78)	(−0.05, 0.75)	(−0.02, 0.82)
Trichlorfon	0.26	−0.07	−0.02
(High vs ND)	(−0.14, 0.66)	(−0.48, 0.34)	(−0.43, 0.39)
Trichlorfon	−0.15	−0.17	−0.11
(Med. vs ND)	(−0.55, 0.25)	(−0.58, 0.23)	(−0.52, 0.31)
2-level (Detect/ND)	**Difference in least square means (95% CI)[c]**		
Chlorpyrifos	0.03	−0.41 *	−0.16
(Detect vs ND)	(−0.31, 0.37)	(−0.75, −0.06)	(−0.51,0.19)
Phorate	−0.09	−0.45 *	−0.28
(Detect vs ND)	(−0.52, 0.33)	(−0.88, −0.01)	(−0.72,0.15)

[a] Models adjusted for sex, age, and cord ferritin

[b] Estimated change in head circumference per 1 unit increase in OP

[c] Difference in mean head circumference

High/Medium/ND cut-offs (ng/mL): methamidophos >18.2/1.5–18.2/ND; trichlorfon >1.7/ 0.4-1.7/ND; chlorpyrifos ≥0.04/ND; phorate ≥1.8/ND

† p<0.10,

* p<0.05