International Journal of Hygiene and Environmental Health 221 (2018) 469–478

Contents lists available at ScienceDirect

International Journal of Hygiene and Environmental Health

journal homepage: www.elsevier.com/locate/ijheh

Prenatal organophosphate insecticide exposure and infant sensory function

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ARTICLE INFO

Keywords:
Pesticide
Chlorpyrifos
Auditory processing
Visual acuity
Neurodevelopment

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 associations between prenatal OP exposure and 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.

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.

1. 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 (USEPA, 2011; Zhang et al., 2011). China is one of the largest consumers of pesticides (Ding and Bao, 2013; USEPA, 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; USCDC, 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

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https://doi.org/10.1016/j.ijheh.2018.01.010
Received 21 August 2017; Received in revised form 29 December 2017; Accepted 18 January 2018
1438-4639/

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 (USEPA, 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.

2. Methods

2.1. 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.

2.2. 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).

2.3. 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 80C 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, 2016). A detailed protocol for the determination of pesticides in cord blood has been described elsewhere (Silver et al., 2016b). Briefly, 800 µL 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 serum 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.

2.4. 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 cd/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