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Prenatal exposure to the herbicide 2,4-D is associated with deficits in auditory processing during infancy

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Abstract

Introduction—Despite widespread use, many herbicides and fungicides are not well studied for neurological effects. Fetal and infant brains are rapidly developing, yet the effects of early-life exposure to these classes of pesticides on visual and auditory function are unknown. Here we examined the effects of prenatal herbicide and fungicide exposure on infant grating visual acuity (VA) and auditory brainstem response (ABR).

Methods—9 herbicides and 13 fungicides were measured in umbilical cord blood plasma from a cohort of infants in Fuyang County, China (n=232). Grating VA and ABR latencies for waves I, III, V were measured at 3 time points: 6 weeks, 9 months, and 18 months. Outcomes included VA score, ABR wave V latency and ABR central conduction time (CCT [wave V- wave I]). Pesticides were analyzed as 3- level ordinal (non-detect [ND]/medium/high), or dichotomous (ND/detect), depending on detection rates. Linear mixed models were used to evaluate relations between pesticides and VA and ABR outcomes.

Results—2,4-dichloroacetic acid (2,4-D), prometryn, simazine, and tetrahydrophthalamide (THPI, a metabolite of captan) were detected in 27, 81, 17, and 16% of samples, respectively. Infants prenatally exposed to 2,4-D had slower auditory response times at 6 weeks. Infants with cord levels of 2,4-D >1.17 ng/mL had wave V latencies that were 0.12 (95% CI: 0.03, 0.22) ms slower (p=0.01) and overall CCTs that were 0.15 (95% CI: 0.05, 0.25) ms slower (p=0.003) than

Human subjects

This study was approved by the institutional review boards at the University of Michigan (HUM00010107) and Zhejiang University (approval # 2011001).

infants with non-detectable 2,4-D in their cord blood. No other statistically significant findings were observed for the other herbicides and fungicides or for the grating VA outcome.

Conclusions—Prenatal exposure to the herbicide 2,4-D was associated with slower auditory signal transmission in early infancy. ABR latencies reflect auditory pathway maturation and longer latencies may indicate delayed auditory development.

Keywords

Fungicides; herbicides; auditory processing; visual acuity; neonates

1. Introduction

China is the world's top producer/exporter of pesticides as well as one of the largest consumers (Ding and Bao, 2013; U.S.EPA, 2011; Zhang et al., 2011). Rates of agricultural pesticide use in China are reported to be nearly five times the global average (Zhang et al., 2014). Rates in Zhejiang province, the site of this study, are thought to be even higher (Huang et al., 2001).

Global herbicide use has risen dramatically over the past several decades (Kniss, 2017; Zhang et al., 2011). While China has been an important producer and exporter during that time, herbicide use in China was minimal until the mid- 2000s (Huang et al., 2017). A reduction in the agricultural workforce, as a result of urbanization, as well as the declining price of herbicides, led farmers increasingly to substitute manual weed control practices with chemical herbicides (Huang et al., 2017). Currently, herbicides make up over one-third of all pesticides used in China (Ding and Bao, 2013).Herbicides, which are used to control the unwanted growth of plants, have a wide variety of applications in agriculture, garden, lawn, pasture, and forest maintenance, and aquaculture. Fungicides make up smaller portion of the pesticide market but also have widespread utility. Fungicides are used to control parasitic fungi and fungal spores in agriculture, especially after harvest, home and garden settings, infection control for humans and animals, and as preservatives in textiles and leather goods. The broad use of these pesticides can lead to human exposure via a number of pathways: ingestion due to consumption of food grown in treated fields or contaminated water, inhalation of dust or spray drift, dermal contact from residential use on lawns, in parks, or other areas (Roberts and Karr, 2012).

Despite nearly ubiquitous exposure in the general population, many of the herbicides and fungicides on the market have not been well studied for developmental effects. Some epidemiologic and toxicological studies provide evidence that early-life exposure to these pesticides potentially negatively impact birth outcomes, disrupt endocrine, reproductive and immune function, and lead to other health problems in childhood. For example, studies of an agricultural region in Minnesota, U.S., found increased odds of birth defects in an area where fungicides and chlorphenoxy herbicides are heavily used (Garry et al., 1996). Additionally, exposure to triazine herbicides, such as atrazine, from municipal drinking water during gestation has been associated with higher rates of intrauterine growth restriction (IUGR) (Munger et al.,1997), small for gestational age (SGA) (Villanueva et al., 2005), and preterm delivery (PTD) (Stayner et al., 2017).

Glyphosate, a well-studied herbicide, has been associated with endocrine disrupting effects in human liver cell lines (Gasnier et al., 2009) and rat testicular cells (Clair et al., 2012) at environmentally relevant doses. Prenatal exposure to the herbicide 2,4-D was associated with shorter anogenital distance in 3-month-old boys (Dalsager et al., 2018).Vinclozilin, a fungicide, and its metabolites can act as agonists or antagonists for a number of steroid receptors (Molina- Molina et al., 2006), and parental use of fungicides for farm application was significantly associated with having a girl (Garry et al., 2002).

Early-life herbicide and fungicide exposure has also been associated with the development of respiratory morbidities such as asthma and allergies. For example, exposure to herbicides during the first year of life was associated with increased odds of an asthma diagnosis before five years of age (Salam et al., 2004). Similarly, parental use of fungicides, herbicides, and more specifically phenoxy herbicides such as 2,4-D, in farming during pregnancy significantly increased the incidence of development of a hay fever allergy development in offspring (Weselak et al., 2007).

Rapidly developing fetal brains may be susceptible to possible long-term effects of prenatal herbicide or fungicide exposure. We were able to find only three studies that reported neurodevelopmental effects following herbicide or fungicide exposure during pregnancy (Garry et al., 2002; Gunieretal., 2017; Mora et al., 2018). Two of the studies looked at prenatal exposure to manganese-based fungicides and found deficits in social-emotional scores in one-year-old girls (Mora et al., 2018) and reduced IQ in 7-year-olds (Gunier et al., 2017). The other study reported that prenatal glyphosate exposure was associated with increased odds of developing attention-deficit/hyperactivity disorder (ADHD) (Garry et al., 2002).

Even less is known about how exposure to herbicides or fungicides may affect sensory functions, such as visual acuity and auditory processing. Proper visual and auditory system development in infancy is imperative for later learning and developmental processes, as it provides the foundation for the acquisition of non-verbal communication and language and reading skills (Algarin et al., 2003; Chonchaiya et al., 2013).

The current study sought to investigate the extent to which prenatal exposure to herbicides and fungicides is associated with visual and auditory function in infancy.

2. Methods

2.1 Study population

Pregnant women, 37–42 weeks gestation, with healthy, uncomplicated, singleton pregnancies, were enrolled into a longitudinal study of iron deficiency and infant neurodevelopment between 2008 and 2011. The women were recruited from the Maternal and Children’s Hospital in Fuyang, a largely rural county within Zhejiang province in southeastern China. Approximately two-thirds of women lived in a rural area, yet few (< 4%) worked in or had a partner who worked in the agricultural sector (Silver et al., 2016). Further details of this study population have been described previously (Silver et al., 2016).

All study protocols were approved by institutional review boards at the University of Michigan and Zhejiang University. Signed, informed consent was obtained prior to participation in the study.

2.2. Herbicides and fungicides

Following delivery, approximately 10 mL of cord blood was collected in a lavender EDTA tube and immediately frozen. Frozen blood samples were transferred on dry ice (twice weekly) from Fuyang to Zhejiang University Children's Hospital in Hangzhou, where they were separated and stored at −80 C. Frozen plasma was later transferred on dry ice to the Institute of Toxicology at Nanjing Medical University for pesticide analysis. Plasma was analyzed for 18 herbicides, 14 fungicides, and 2 fungicide metabolites 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). The protocol for the determination of 96 pesticides in umbilical cord plasma has been described previously (Silver et al., 2016). Briefly, 800μL plasma samples were mixed with 800μL saturated ammonium sulfate and centrifuged. Following centrifugation, supernatants were subjected to solid phase extraction for cleaning and pre-concentration. Analytes were eluted in dichloromethane and n-Hexane, concentrated, and reconstituted in 10μL toluene prior to GC-MS/MS analysis. Limits of detection (LODs) were determined by analyzing fortified serum on a signal-to-noise ratio of three. Quality control samples were generated using 0.675 or 1.35 ng/mL of pesticide standards. Quality control samples and blanks were analyzed alongside samples.

2,4-D and prometryn were detected in 27.2 and 81.0 % of samples, respectively, and were treated as 3-level ordinal variables for analysis (<LOD, medium, high [median split for those above LOD]; cut-offs were <0.51, 0.51–1.17, >1.17 ng/mL and <0.02, 0.02–6.09, >6.09 ng/mL, for 2,4-D and prometryn, respectively). Simazine and THPI were detected in 17.2 and 15.5% of samples, respectively, and were treated as dichotomous (<LOD/detect; cut-offs were <0.25, ≥0.25 ng/mL and <0.34, ≥0.34 ng/mL, for simazine and THPI, respectively).

2.3 Grating Visual Acuity (VA)

Grating VA improves throughout in infancy and childhood visual pathway matures and myelinates (Tau and Peterson, 2010). For this study, we estimated grating VA using a preferential looking procedure with Teller acuity cards (TAC). The TAC test provides a quantitative measure of binocular grating acuity for infants and nonverbal children. Grating VA was measured three times during infancy, at 6 weeks, 9 months, and 18 months of age.

The details of the TAC procedure used for this study have been described previously (Silver et al., 2018). Briefly, infants were held upright by study staff while facing a TAC stage where they were presented with a series of cards with black and white vertical gratings on one side and a gray "blank" on the other side. Gratings ranged from coarse (0.44 cycles/degree) to fine (27 cycles/degree) and were presented in descending order, with the coarsest gratings presented first; gratings were presented on the left and right sides of the print to avoid habituation. Examiners observed infant looking behavior through an opening in the TAC stage, repeating TAC card presentation until consistent looking toward the location of

the grating occurred. The spatial frequency of the finest grating that the infant could resolve was recorded as the grating VA score.

2.4 Auditory Brainstem Response (ABR)

ABR measures electrical activity of neurons along the auditory pathway following an auditory stimulus. ABRs in infants consist of three main 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). ABR latencies decrease throughout infancy, indicative of faster auditory signal transmission, thus corresponding to the maturation and myelination of the auditory pathway (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. The specific details of the ABR procedure have been described previously (Silver et al., 2018). Briefly, infants underwent a standard hearing screening protocol in both ears. After passing the hearing screen, infants continued on to ABR testing. ABR stimuli were square wave rarefaction monophasic clicks delivered to each ear by insert transducers (presentation rate=11.7/second, duration=100 μs, intensity=80 dB, nHL, recording epoch=74.67 ms). ABRs were recorded by silver/silver chloride electrodes attached to infant's forehead at the midline below the hairline, mastoid on ipsilateral mastoid, and contralateral mastoid (impedance<10 kΩ). For each ear, two successive runs (1300 sweeps/run) were averaged, for a total of 2600 sweeps per ear. Right and left ears were then averaged, 5200 total sweeps, to give a single average ABR waveform for each infant.

Latencies for peaks I, III, and V were extracted from the ABR waveforms. The outcomes of interest for this study were wave V latency and central conduction time (CCT), which is the inter-peak latency from wave I to wave V. These two outcomes are commonly used to represent overall auditory processing abilities because they are easily identifiable and reproducible (Berglund et al., 2011). Further, wave V latencies have historically been used as a measure of auditory system integrity (Hecox and Galambos, 1974).

2.5 Covariates

Infant sex (male/female) was recorded at birth. Infant age in days was obtained at the time of auditory and visual testing (approximately 6 weeks, 9 months, and 18 months). Serum ferritin was measured by chemiluminescent immunoassay (IMMULITE, Diagnostic Products) in cord blood and again at 9 months of age. Iron deficiency at birth was defined as cord serum ferritin (SF) ≤75 μg/L. Very high values (SF >370 μg/L) were excluded, as they indicate the presence of infection or inflammation. Iron deficiency at 9 months was defined as two or more of the following measurements: mean corpuscular volume (MCV) <74 fl, red cell distribution width (RDW) >14.5%, SF <12.0 μg/L, zinc protoporphyrin/heme (ZPP/H) >69 μmol/mol. Birth weight in grams was recorded at birth. Head circumference was measured at the 6-week, 9-month, and 18-month visits using a soft tape placed wrapped around the widest part of the head. Two measurements were taken and the average of the two was recorded. Yearly family income, maternal education and occupation were obtained

by maternal self-report from a questionnaire administered at the 6-week visit and subsequently converted to categorical variables for analysis; family income (<30,000/30,000–49,999/50,000– 99,999/≥100,000 Yuan), maternal education (middle school or less/high school or secondary school/college), maternal occupation (housewife/other).

2.6 Statistical analysis

Statistical analyses were conducted using SAS 9.4 (Cary, North Carolina). Descriptive statistics and frequencies were examined for all covariates of interest.

Linear mixed models (LMM) with random intercepts were used to evaluate associations between cord pesticide exposures and grating VA scores and ABR latencies (wave V latency, CCT) during infancy. Age was centered at 6 weeks. Model covariates were chosen based on several criteria. Infant sex, age at testing, and iron status were chosen *a priori.* Additional covariates considered for inclusion were gestational age, birth weight, head circumference, family income, maternal education and occupation. Final models were selected based on biological plausibility, overall model fit, and whether the pesticide parameter estimates were changed by ≥10% with inclusion/exclusion of the variable. We selected two final models. The most parsimonious, model 1, was minimally adjusted for sex, age at testing, and iron status (6 weeks, 9 months). Model 2 was additionally adjusted for birth weight, head circumference (6 weeks, 9 months, 18 months), and family income. To identify potential sexually dimorphic effects, we also ran these models stratified by infant sex. We alternatively examined the longitudinal models using a least squares means (LS means) approach. This approach yields easily interpretable estimates of means and differences in means for each category of exposure at the three time points by including and “time” as a class variable and “time*pesticide” in the models.

3. Results

Nine herbicides and 13 fungicides were detected in the cord blood plasma of our study population. Their distributions are shown in Table 1.

Only four were detected in at least 10% of samples. 2,4-D, prometryn, simazine, and THPI were detected in 27.2, 81.0, 17.2, and 15.5% of samples, respectively. The mean (SD) of herbicides and fungicides detected per cord blood sample was 1.3 (0.7) and 0.2 (0.7), for herbicides and fungicides, respectively. 31 % of infants had detectable levels of two or more herbicides, while only 3% had detectable levels of two or more fungicides. Infant neurodevelopmental testing was available for 232 infants with pesticide data. Characteristics of the study sample are shown in Table 2.

Further characteristics of the study population have been reported previously (Silver et al., 2016). Mean (SD) grating VA scores were 1.17 (0.54), 7.67 (2.34), and 9.45 (2.58) at 6 weeks, 9 months, and 18 months, respectively. Grating VA scores were not correlated across time points; Spearman correlations were 0.03 (p=0.74), −0.01 (p=0.95), and 0.10 (p=0.22) for VA at 6 weeks/9 months, 6 weeks/18 months and 9 months/18 months, respectively. Mean (SD) wave V ABR latencies were 6.45 (0.26), 5.90 (0.26), and 5.71 (0.23) ms and the

ABR CCTs were 4.94 (0.27), 4.40 (0.25), 4.20 (0.21) ms, at 6 weeks, 9 months, and 18 months, respectively. ABR wave V and CCT outcomes were highly correlated across time points with ρ's ranging from 0.69 to 0.93 ($p<0.0001$).

3.1 ABR

Adjusted LMM results for the effect of prenatal pesticide exposure on ABR outcomes, wave V latency and CCT, are shown in Table 3.

Prenatal exposure to 2,4-D was significantly associated with slower auditory signal transmission. Infants prenatally exposed to high levels of 2,4-D (>1.17 ng/mL) had wave V latencies that were 0.12 (95% Cl: 0.03, 0.22) ms slower ($p=0.01$) at 6 weeks than infants with non-detectable 2,4-D in their cord blood. CCTs were 0.15 (95% Cl: 0.05, 0.25) ms slower ($p=0.003$) and 0.04 (95% Cl: −0.07, 0.14) ms slower at 6 weeks for infants with high and medium 2,4-D exposure, respectively; p-trend=0.004. After re-running the models with age centered at 9 months and 18 months, we determined that prenatal 2,4-D exposure had no significant effect on ABR outcomes at the two later time points (data not shown). Prometryn, simazine, and THPI were not significantly associated with the ABR outcomes at any exposure level for any of the time points. Additional model estimates for intercepts, age at testing, interaction terms, etc. were omitted from Table 3 for the sake of simplicity, though briefly summarize one additional key finding here. The 2,4-D LMM models also estimated that, infants' wave V latencies, on average, would decrease by 0.0050 ms, for each day older, when prenatal 2,4-D exposure was <LOD, but infants with high prenatal 2,4-D exposure wave V latencies' would decrease slightly faster, by 0.0052 ms ($p=0.03$), for each day older. There were no other statistically significant differences in wave V or CCT slopes by prenatal exposure level for any of the other pesticides examined.

Sex-stratified models (Table 3) revealed no major differences in effect size by sex. For example, wave V latencies were 0.13 (95% Cl: −0.03, 0.29) ms slower for boys with high prenatal 2,4-D exposure compared to those with non- detectable 2,4-D and 0.14 (0.02, 0.26) ms slower for girls with high prenatal 2,4-D exposure compared to those with non-detectable 2,4-D. Results were slightly stronger for girls compared to boys; p-values were 0.09 and 0.02, for boys and girls, respectively, and tests for linear trend were statistically significant for girls but not boys; p-trend=0.03 and 0.02 for girls' wave V and CCT latencies, respectively.

The LS means procedure yielded similar effect estimates. Infants exposed prenatally to 2,4-D had ABR wave V latencies that were 0.13 (0.03, 0.22) ms longer ($p=0.007$) and CCTs that were 0.15 (0.05, 0.25) ms longer ($p=0.002$), at 6 weeks, compared to infants with 2,4-D levels below the LOD. There were no statistically significant differences between exposure groups at 9 months or 18 months (Figures 1 [ABR] and 2 [CCT]).

3.2 Grating VA

Adjusted LMM results for grating VA score are shown in Table 4.

In general, concentrations of herbicides and fungicides in cord blood were associated with slightly lower grating VA scores in infancy, though these results were not statistically

significant. Sex-stratified models (Table 4) similarly revealed no major differences in effect size by sex. The LS means procedure similarly yielded insignificant results (Figure 3).

4. Discussion

We found that infants prenatally exposed to the herbicide 2,4-D had slower auditory transmission early in infancy, compared to infants with lower 2,4-D exposure or exposure below the LOD. This effect seemed to be attenuated by the later time points. The other herbicides and fungicides examined were not associated with ABR outcomes. Grating VA scores tended to be lower in herbicide and fungicide exposed infants, though results were not statistically significant. Sex- specific analyses did not reveal any significant differences in effect size between male or female infants, though tests for linear trend were statistically significant for the ABR outcomes in females only.

Interestingly, the rate of decrease in wave V latency was significantly faster (more negative) in infants exposed to high levels of 2,4-D prenatally versus those with exposure <LOD. Initially this was surprising because it appeared as though the auditory pathways of infants with the highest 2,4-D exposures may be maturing at a slightly faster rate than infants with low exposures. However, further examination revealed that the differences in slope are simply a result of the significantly longer wave V latencies observed for high 2,4-D-exposed infants at 6 weeks, coupled with no significant difference in latencies at 9 and 18 months.

To our knowledge this is the first study to report 2,4-D, prometryn, or simazine levels in cord blood. Cooper, et al. attempted to measure both 2,4-D and simazine in cord blood but did not find detectable levels of either (Cooper et al.,2001). The highest levels of THPI found in the current study were about 25 to 70 times higher than the maximum levels previously reported in cord serum of infants in the U.S. The maximum concentration of THPI found in cord blood in the current study was 1.02 ng/mL, compared to maximums of 0.014 ng/mL (Yan et al., 2009) and ~0.038 ng/mL (Whyatt et al., 2003) in U.S. infants.

2,4-D is a phenoxy herbicide in use since the 1940s. It is registered for use in the U.S., China and elsewhere and is still one of the most highly used herbicides in the world (Islam et al., 2018). 2,4-D is used on fruit, vegetable and field crops, turf and lawns, and aquatic and forestry sites to combat the growth and spread of broadleaf weeds (Islam et al., 2018). 2,4-D can also be utilized to regulate the growth of citrus plants. In China, 5,000 to 8,000 tons of 2,4-D butyl ester/ butylate are applied to wheat, soybean, corn and other crops in China each year (Islam et al., 2018). China's new list of maximum residue limits for food, released in 2017, includes limits for 2,4-D and 2,4-D butylate for a wide variety of cereal grains, oil, vegetables and fruits (GAIN, 2017). In the U.S., studies of pesticide exposure in preschool-aged children revealed the presence of 2,4-D in soil, indoor and outdoor air, carpet dust, solid and liquid food, hand wipes, and spot urine samples (Morgan et al., 2008). Last year, the U.S. Environmental Protection Agency approved expanded usage of Enlist Duo, an herbicide mixture consisting of 2,4-D and glyphosate, to include corn, soybean and cotton crops. This expansion is expected to increase the use of 2,4-D in the U.S. two- to six-fold by 2020 (USDA, 2013).

Information about the effects of fungicides or herbicides on human neurodevelopment is quite limited. To our knowledge this is the first human study of prenatal exposure to prometryn, simazine, or THPI and neurodevelopment. We were able to find one study that examined prenatal 2,4-D exposure and ADHD in childhood. There was no association observed between farm families' reported use of chlorphenoxy herbicides, including 2,4-D, during pregnancy and the development of ADHD in their offspring (Garry et al., 2002). We were unable to find any studies that looked specifically at the effects of prenatal herbicide or fungicide exposure and visual or auditory neurodevelopment.

A few studies have examined prenatal exposure to other herbicides and fungicides, different from those examined in the current study, and neurodevelopmental outcomes. Garry and colleagues found increased odds of having a child with ADHD among parents who reported using the herbicide glyphosate during pregnancy (Garry, 2002). Additionally, there are several studies of prenatal exposure to manganese based/containing fungicides and neurodevelopment. Prenatal exposure to mancozeb was associated with decreased Bayley's social-emotional scores in 1-year-old girls (Mora et al., 2018), while residential proximity to agricultural use of maneb and mancozeb during gestation was associated with reduced IQ in 7-year-old children (Gunier et al., 2017). Similarly, 6- to 9-year-old children with higher concurrent mancozeb exposure had poorer verbal learning outcomes (van Wendel de Joode et al., 2016).

The occupational literature regarding visual or auditory effects of 2,4-D is also scarce. One study found that use of phenoxyacetate herbicides, including 2,4-D, was associated with higher odds of age-related macular degeneration in farmers in the Agricultural Health Study (U.S.) (Montgomery et al., 2017). To our knowledge, no auditory effects of 2,4-D exposure have been reported in farmers or pesticide applicators. However, other herbicides, such as paraquat, have been associated with damage to outer and inner hair cells of the ear (Bielefeld et al., 2005; Nicotera et al., 2004; Zhang et al., 2018), as well as the dislocation and apoptosis of their support cells (Zhang et al., 2018).

In the current study we found that infants prenatally exposed to the herbicide 2,4-D had slower auditory processing abilities. One potential mechanism by which 2,4-D may be contributing to longer ABR latencies in exposed infants is via the disruption of myelination. ABR latencies in infancy are directly correlated to the level of myelination of the auditory pathway. ABR latencies decrease and signal transmission speeds increase throughout infancy as the auditory pathway matures and becomes fully myelinated (Hecox and Galambos, 1974; Jiang, 1995). Several toxicology studies support the hypothesis that 2,4-D may negatively impact myelination. Rat pups exposed postnally, during periods of rapid central nervous system (CNS) myelination, had significantly lower levels of myelin deposition (Rosso et al., 2000), significantly less expression of myelin-specific proteins, myelin compaction, and number of myelin sheets (Konjuh et al., 2008), and significant deficits in myelin markers and myelin volume in the brain (Duffard et al., 1996), when compared with unexposed controls. These studies indicate that 2,4-D exposure, during sensitive periods of brain growth, may negatively impact myelination, which could potentially affect the maturation and function of the auditory pathway.

Another potential mechanism for how 2,4-D exposure may contribute to longer ABR latencies is disruption of dopaminergic signaling along the auditory pathway. Toxicological studies reveal that 2,4-D affects dopamine (DA) levels, dopaminergic signaling pathways, and DA receptors in the brains of rats (Bortolozzi et al., 2003; Bortolozzi et al., 1998; Bortolozzi et al., 2004). Researchers recently discovered DA release in the inferior colliculus (IC) of the brain (Batton et al., 2018). The IC is an important convergence point for the ascending and descending auditory pathways (Batton et al., 2018), indicating that DA may play an important role in central auditory processing. It is plausible that a disruption of DA signaling could potentially interfere with the auditory pathway via the IC. Interestingly, human occupational exposure to 2,4-D has been associated with an increased risk of Parkisonsinism among agricultural workers (Tanner et al., 2009). Parkinson's disease results in a loss of DA, and it is common for patients to present with deficits in auditory processing, which are often unrelated to age-related hearing loss (Batton et al., 2018).

Our study is limited in several ways. The pesticides studied here were non-persistent with short half-lives in the environment and the body. For example, 2,4-D has a biological half-life of 10–33 hours (Burns and Swaen, 2012). Therefore, having only one measure of exposure, at birth, limited our ability to address the temporal variability of the exposure during pregnancy/infancy and may have led us to miss some exposures at sensitive stages of development (Eskenazi et al., 2007). The laboratory methods used here were not optimized for detection of these particular pesticides, but rather were part of a large semi-targeted analysis of 96 pesticides and metabolites from a broad range of classes (Silver et al., 2016). This semi-targeted approach likely resulted in higher detection limits and more non-detects, compared to what may have been seen for a more targeted method. Pesticide levels in blood tend to be lower anyway, compared to urine metabolites, which may also have contributed to the number of non-detects (Barr et al., 1999). Additionally, there are several formulations of 2,4-D, with the acid, ester, and salt forms being the most common. The analytical methods used here were limited to measurement of the acid form only. 2,4-D esters are degraded into the acid form once they are absorbed into the body (Garabrant and Philbert, 2002). Thus, our methods should have been able to quantify exposure to both the acid and ester forms, which are the most commonly used forms in China (Islam et al.,2018). It is possible that we missed some 2,4-D exposure, if it was from one of the less commonly used 2,4-D formulations. Furthermore, our modest sample size, coupled with relatively low detection rates for our pesticides, necessitated the use of single pollutant models, thereby limiting our ability to assess the effects of pesticide mixtures. Our hospital staff was highly trained, but assessing infants at such young ages increases the chance of error, especially for the grating VA test. Finally, the findings from this 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 born at term without health complications. Pre-term or low birth weight infants are more likely to have experience developmental delays, and the effect of 2,4-D and other pesticides on these vulnerable populations should also be investigated. Finally, because this was not originally designed as an environmental exposure study, we do not have information about possible sources of prenatal 2,4-D exposure, such as maternal diet during pregnancy or proximity to agriculture.

Despite its limitations, this study has notable strengths. The measurement of parent compounds in umbilical cord blood to assign prenatal exposure, rather than non-specific urinary metabolites, provides direct evidence of fetal exposure (Barr et al., 1999; Munoz-Quezada et al., 2013). Additionally, levels in cord blood may more accurately represent the available dose, since the pesticides have not yet been eliminated from the body (Needham et al., 1995). Thus, the use of cord blood to define exposure more precisely increases the utility of these findings for policy makers. This is the first study to quantify the levels of many of these pesticides in cord blood. To our knowledge, this is also the first human study of prenatal exposure to the majority of these pesticides and any neurodevelopment-related outcome. Only one other study has examined associations between parental use of chlorphenoxy herbicides (2,4-D) during pregnancy and neurodevelopment (ADHD), though it was limited by its use of a phone survey for exposure assessment, as well as limited diagnostic criteria for the neurobehavioral follow-up (Garry et al., 2002). The current study is also the first to look specifically at the effects of prenatal herbicide or fungicide exposure and visual or auditory development. ABR and grating VA provide non-invasive ways of measuring auditory and visual development and function over the course of infancy. Our longitudinal assessment of sensory development over three time points in infancy (6 weeks, 9 months, and 18 months) gives a comprehensive view of overall visual and auditory development.

Conclusions

We found that infants with prenatal exposure to the commonly used herbicide 2,4-D had significantly slower auditory signal transmission during early infancy. The clinical importance of these small, yet statistically significant, deficits in auditory processing at such an early age are unclear, yet warrant further study given the widespread use of 2,4-D globally. In the current study, the significant effect of prenatal 2,4-D exposure on auditory processing in 6-week-olds was attenuated at 9 and 18 months; however, it is unknown if further follow-up would reveal any long-term changes in auditory processing or related functions. The proper maturation of the auditory pathway during infancy is crucial for later cognitive processes. Perturbation of this early stage of neurodevelopment may have the potential for negative effects on learning later in childhood. Future work should include a follow-up of these children at later stages of development to assess the possible long-term effects of prenatal pesticide exposure, as well as an exploration of these associations in additional, independent populations.

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References

Algarin C, Peirano P, Garrido M, Pizarro F, Lozoff B, 2003 Iron deficiency anemia in infancy: long-lasting effects on auditory and visual system functioning. Pediatr Res 53, 217–223. [PubMed: 12538778]

Barr DB, Barr JR, Driskell WJ, Hill RH Jr., Ashley DL, Needham LL, Head SL, Sampson EJ, 1999 Strategies for biological monitoring of exposure for contemporary-use pesticides. Toxicol Ind Health 15, 168–179. [PubMed: 10188199]

Batton AD, Blaha CD, Bieber A, Lee KH, Boschen SL, 2018 Stimulation of the subparafascicular thalamic nucleus modulates dopamine release in the inferior colliculus of rats. Synapse.

Berglund SK, Westrup B, Haraldsson E, Engstrom B, Domellof M, 2011 Effects of iron supplementation on auditory brainstem response in marginally LBW infants. Pediatr Res 70, 601–606. [PubMed: 21857390]

Bielefeld EC, Hu BH, Harris KC, Henderson D, 2005 Damage and threshold shift resulting from cochlear exposure to paraquat-generated superoxide. Hear Res 207, 35–42. [PubMed: 15935579]

Bortolozzi A, Duffard R, de Duffard AM, 2003 Asymmetrical development of the monoamine systems in 2,4- dichlorophenoxyacetic acid treated rats. Neurotoxicology 24, 149–157. [PubMed: 12564390]

Bortolozzi A, Duffard RO, Rubio M, Sturtz N, Evangelista de Duffard AM, 1998 Regionally specific changes in central brain monoamine levels by 2,4-dichlorophenoxyacetic acid in acute treated rats. Neurotoxicology 19, 839–851. [PubMed: 9863773]

Bortolozzi AA, Evangelista De Duffard AM, Duffard RO, Antonelli MC, 2004 Effects of 2,4-dichlorophenoxyacetic acid exposure on dopamine D2-like receptors in rat brain. Neurotoxicol Teratol 26, 599–605. [PubMed: 15203183]

Burns CJ, Swaen GM, 2012 Review of 2,4-dichlorophenoxyacetic acid (2,4-D) biomonitoring and epidemiology. Crit Rev Toxicol 42, 768–786. [PubMed: 22876750]

Chonchaiya W, Tardif T, Mai X, Xu L, Li M, Kaciroti N, Kileny PR, Shao J, Lozoff B, 2013 Developmental trends in auditory processing can provide early predictions of language acquisition in young infants. Dev Sci 16, 159–172. [PubMed: 23432827]

Clair E, Mesnage R, Travert C, Seralini GE, 2012 A glyphosate-based herbicide induces necrosis and apoptosis in mature rat testicular cells in vitro, and testosterone decrease at lower levels. Toxicol In Vitro 26, 269–279. [PubMed: 22200534]

Cooper SP, Burau K, Sweeney A, Robison T, Smith MA, Symanski E, Colt JS, Laseter J, Zahm SH, 2001 Prenatal exposure to pesticides: a feasibility study among migrant and seasonal farmworkers. Am J Ind Med 40, 578–585. [PubMed: 11675627]

Dalsager L, Christensen LE, Kongsholm MG, Kyhl HB, Nielsen F, Schoeters G, Jensen TK, Andersen HR, 2018 Associations of maternal exposure to organophosphate and pyrethroid insecticides and the herbicide 2,4-D with birth outcomes and anogenital distance at 3 months in the Odense Child Cohort. Reprod Toxicol 76, 53–62. [PubMed: 29274948]

DeBonis DA, Donohue CL, 2008 Survey of Audiology: Fundamentals for Audiologists and Health Professionals (2nd ed.). Allyn & Bacon, Boston.

Ding G, Bao Y, 2013 Revisiting pesticide exposure and children's health: Focus on China. Sci Total Environ 472C, 289–295.

Duffard R, Garcia G, Rosso S, Bortolozzi A, Madariaga M, di Paolo O, Evangelista de Duffard AM, 1996 Central nervous system myelin deficit in rats exposed to 2,4-dichlorophenoxyacetic acid throughout lactation. Neurotoxicol Teratol 18, 691–696. [PubMed: 8947946]

Eskenazi B, Marks AR, Bradman A, Harley K, Barr DB, Johnson C, Morga N, Jewell NP, 2007 Organophosphate pesticide exposure and neurodevelopment in young Mexican-American children. Environ Health Perspect 115, 792–798. [PubMed: 17520070]

GAIN, 2017 China Releases New Maximum Residue Limits for Pesticides in Food, in: Service UFA (Ed.), Global Agricultural Information Network. United States Department of Agriculture, Beijing.

Garabrant DH, Philbert MA, 2002 Review of 2,4-dichlorophenoxyacetic acid (2,4-D) epidemiology and toxicology. Crit Rev Toxicol 32, 233–257. [PubMed: 12184504]

Garry VF, Harkins ME, Erickson LL, Long-Simpson LK, Holland SE, Burroughs BL, 2002 Birth defects, season of conception, and sex of children born to pesticide applicators living in the Red River Valley of Minnesota, USA. Environ Health Perspect 110 Suppl 3, 441–449. [PubMed: 12060842]

Garry VF, Schreinemachers D, Harkins ME, Griffith J, 1996 Pesticide appliers, biocides, and birth defects in rural Minnesota. Environ Health Perspect 104, 394–399. [PubMed: 8732949]

Gasnier C, Dumont C, Benachour N, Clair E, Chagnon MC, Seralini GE, 2009 Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. Toxicology 262, 184–191. [PubMed: 19539684]

Gunier RB, Bradman A, Harley KG, Kogut K, Eskenazi B, 2017 Prenatal Residential Proximity to Agricultural Pesticide Use and IQ in 7-Year-Old Children. Environ Health Perspect 125, 057002. [PubMed: 28557711]

Hall JI, 2007 The new handbook of auditory evoked responses. Allyn & Bacon, Boston.

Hecox K, Galambos R, 1974 Brain stem auditory evoked responses in human infants and adults. Arch Otolaryngol 99, 30–33. [PubMed: 4809084]

Huang J, Qiao F, Zhang L, Rozelle S, 2001 Farm pesticide, rice production, and human health. Economy and Environment Program for Southeast Asia, Singapore.

Huang J, Wang S, Xiao Z, 2017 Rising Herbicide Use and Its Driving Forces in China. The European Journal of Development Research 29, 614–627.

Islam F, Wang J, Farooq MA, Khan MSS, Xu L, Zhu J, Zhao M, Munos S, Li QX, Zhou W, 2018 Potential impact of the herbicide 2,4-dichlorophenoxyacetic acid on human and ecosystems. Environ Int 111, 332–351. [PubMed: 29203058]

Jiang ZD, 1995 Maturation of the auditory brainstem in low risk-preterm infants: a comparison with age-matched full term infants up to 6 years. Early Hum Dev 42, 49–65. [PubMed: 7671845]

Kniss AR, 2017 Long-term trends in the intensity and relative toxicity of herbicide use. Nat Commun 8, 14865. [PubMed: 28393866]

Konjuh C, Garcia G, Lopez L, de Duffard AM, Brusco A, Duffard R, 2008 Neonatal hypomyelination by the herbicide 2,4-dichlorophenoxyacetic acid. Chemical and ultrastructural studies in rats. Toxicol Sei 104, 332–340.

Molina-Molina JM, Hillenweck A, Jouanin I, Zalko D, Cravedi JP, Fernandez MF, Pillon A, Nicolas JC, Olea N, Balaguer P, 2006 Steroid receptor profiling of vinclozolin and its primary metabolites. Toxicol Appl Pharmacol 216, 44–54. [PubMed: 16750840]

Montgomery MP, Postei E, Umbach DM, Richards M, Watson M, Blair A, Chen H, Sandler DP, Schmidt S, Kamel F, 2017 Pesticide Use and Age-Related Macular Degeneration in the Agricultural Health Study. Environ Health Perspect 125, 077013. [PubMed: 28886597]

Mora AM, Cordoba L, Cano JC, Hernandez-Bonilla D, Pardo L, Schnaas L, Smith DR, Menezes-Filho JA, Mergler D, Lindh CH, Eskenazi B, van Wendel de Joode B, 2018 Prenatal Mancozeb Exposure, Excess Manganese, and Neurodevelopment at 1 Year of Age in the Infants' Environmental Health (ISA) Study. Environ Health Perspect 126, 057007. [PubMed: 29847083]

Morgan MK, Sheldon LS, Thomas KW, Egeghy PP, Croghan CW, Jones PA, Chuang JC, Wilson NK, 2008 Adult and children's exposure to 2,4-D from multiple sources and pathways. J Expo Sei Environ Epidemiol 18, 486–494.

Munger R, Isacson P, Hu S, Burns T, Hanson J, Lynch CF, Cherryholmes K, Van Dorpe P, Hausier WJ Jr., 1997 Intrauterine growth retardation in Iowa communities with herbicide-contaminated drinking water supplies. Environ Health Perspect 105, 308–314. [PubMed: 9171992]

Munoz-Quezada MT, Lucero BA, Barr DB, Steenland K, Levy K, Ryan PB, Iglesias V, Alvarado S, Concha C, Rojas E, Vega C, 2013 Neurodevelopmental effects in children associated with exposure to organophosphate pesticides: a systematic review. Neurotoxicology 39, 158–168. [PubMed: 24121005]

Needham LL, Ashley DL, Patterson DG Jr., 1995 Case studies of the use of biomarkers to assess exposures. Toxicol Lett 82–83, 373–378.

Nicotera TM, Ding D, McFadden SL, Salvemini D, Salvi R, 2004 Paraquat-induced hair cell damage and protection with the superoxide dismutase mimetic m40403. Audiol Neurootol 9, 353–362. [PubMed: 15467288]

Roberts JR, Karr CJ, 2012 Pesticide exposure in children. Pediatrics 130, e1765–1788. [PubMed: 23184105]

Rosso SB, Garcia GB, Madariaga MJ, Evangelista de Duffard AM, Duffard RO, 2000 2,4-Dichlorophenoxyacetic acid in developing rats alters behaviour, myelination and regions brain gangliosides pattern. Neurotoxicology 21, 155–163. [PubMed: 10794395]

Salam MT, Li YF, Langholz B, Gilliland FD, 2004 Early-life environmental risk factors for asthma: findings from the Children's Health Study. Environ Health Perspect 112, 760–765. [PubMed: 15121522]

Silver MK, Shao J, Chen M, Xia Y, Lozoff B, Meeker JD, 2016 Distribution and Predictors of Pesticides in the Umbilical Cord Blood of Chinese Newborns. Int J Environ Res Public Health 13.

Silver MK, Shao J, Ji C, Zhu B, Xu L, Li M, Chen M, Xia Y, Kaciroti N, Lozoff B, Meeker JD, 2018 Prenatal organophosphate insecticide exposure and infant sensory function. Int J Hyg Environ Health 221, 469–478. [PubMed: 29402694]

Stayner LT, Almberg K, Jones R, Gräber J, Pedersen M, Turyk M, 2017 Atrazine and nitrate in drinking water and the risk of preterm delivery and low birth weight in four Midwestern states. Environ Res 152, 294–303. [PubMed: 27816866]

Tanner CM, Ross GW, Jewell SA, Hauser RA, Jankovic J, Factor SA, Bressman S, Deligtisch A, Marras C, Lyons KE, Bhudhikanok GS, Roucoux DF, Meng C, Abbott RD, Langston JW, 2009 Occupation and risk of parkinsonism: a multicenter case-control study. Arch Neurol 66, 1106–1113. [PubMed: 19752299]

Tau GZ, Peterson BS, 2010 Normal development of brain circuits. Neuropsychopharmacology 35, 147–168. [PubMed: 19794405]

U.S.EPA, 2011 Pesticide industry sales and usage: 2006 and 2007 market estimates. US Environmental Protection Agency, Washington, DC.

USDA, 2013 Dow AgroSciences Petitions (09–233-01 p, 09–349-01 p, and 11 −234–01 p) for Determinations of Nonregulated Status for 2,4-D-Resistant Corn and Soybean Varieties Draft Environmental Impact Statement. United States Department of Agriculture.

van Wendel de Joode B, Mora AM, Lindh CH, Hernandez-Bonilla D, Cordoba L, Wesseling C, Hoppin JA, Mergler D, 2016 Pesticide exposure and neurodevelopment in children aged 6–9 years from Talamanca, Costa Rica. Cortex 85, 137–150. [PubMed: 27773359]

Villanueva CM, Durand G, Coutte MB, Chevrier C, Cordier S, 2005 Atrazine in municipal drinking water and risk of low birth weight, preterm delivery, and small-for-gestational-age status. Occup Environ Med 62, 400–405. [PubMed: 15901888]

Weselak M, Arbuckle TE, Wigle DT, Krewski D, 2007 In utero pesticide exposure and childhood morbidity. Environ Res 103, 79–86. [PubMed: 17084836]

Whyatt RM, Barr DB, Camann DE, Kinney PL, Barr JR, Andrews HF, Hoepner LA, Garfinkel R, Hazi Y, Reyes A, Ramirez J, Cosme Y, Perera FP, 2003 Contemporary-use pesticides in personal air samples during pregnancy and blood samples at delivery among urban minority mothers and newborns. Environ Health Perspect 111, 749–756. [PubMed: 12727605]

Yan X, Lashley S, Smulian JC, Ananth CV, Barr DB, Ledoux TA, Hore P, Robson MG, 2009 Pesticide concentrations in matrices collected in the perinatal period in a population of pregnant women and newborns in New Jersey, USA. Human and Ecological Risk Assessment: An International Journal 15, 948–967.

Zhang J, Sun H, Salvi R, Ding D, 2018 Paraquat initially damages cochlear support cells leading to anoikis-like hair cell death. Hear Res 364, 129–141. [PubMed: 29563067]

Zhang W, Jiang F, JF. O, 2011 Global pesticide consumption and pollution: with China as a focus. Proceedings of the International Academy of Ecology and Environmental Sciences 1, 125–144.

Zhang Y, Han S, Liang D, Shi X, Wang F, Liu W, Zhang L, Chen L, Gu Y, Tian Y, 2014 Prenatal exposure to organophosphate pesticides and neurobehavioral development of neonates: a birth cohort study in shenyang, china. PLoS One 9, e88491. [PubMed: 24551109]

Highlights

- 22 herbicides and fungicides were measured in umbilical cord blood
- Auditory (ABR) and visual (grating VA) function were measured 3 times in infancy
- Prenatal 2,4-D exposure was associated with slower auditory processing at 6 weeks
- Longer ABR latencies may indicate delayed development of auditory pathway

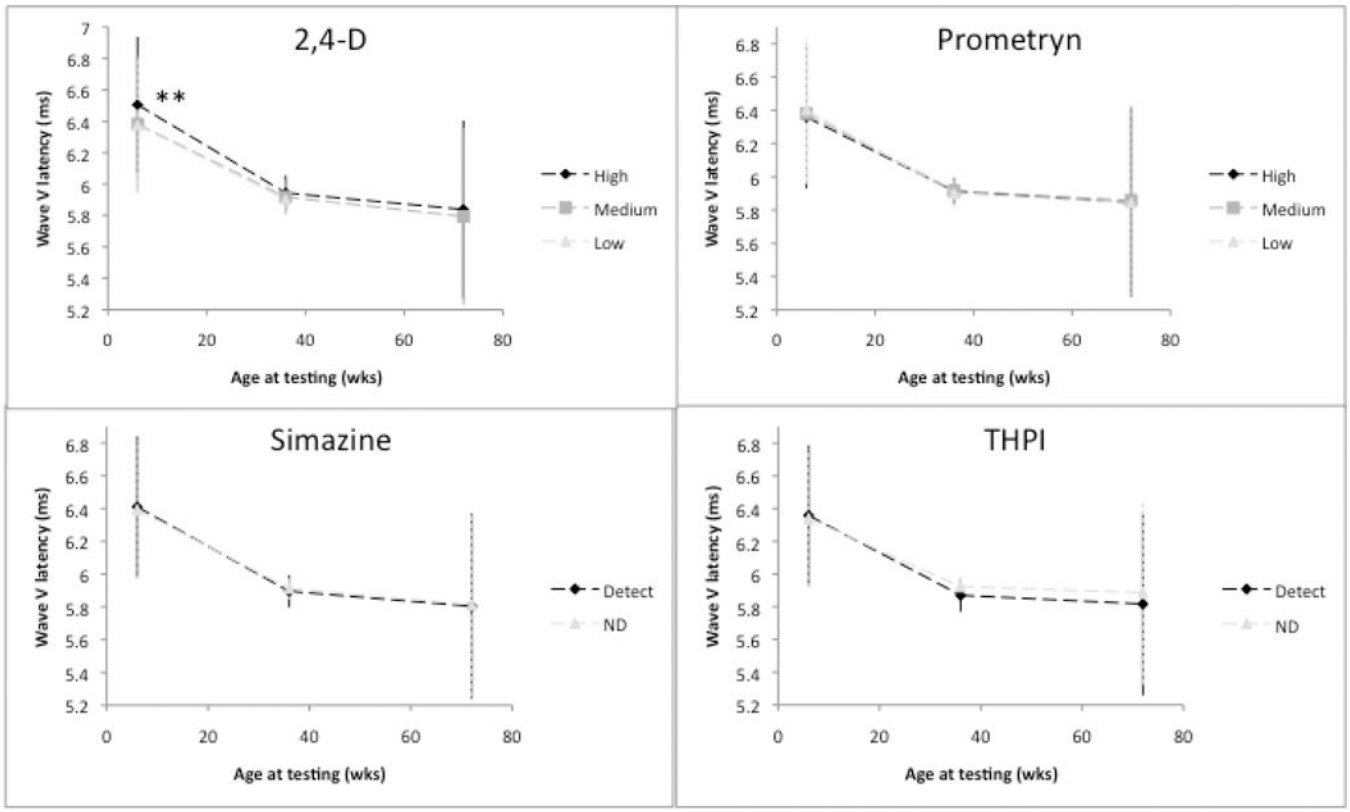

Fig 1.

Adjusted least squares means for the effect of prenatal pesticide exposure on infant ABR wave V latencies Mixed effects models with random intercepts; Adjusted for sex, age at testing, iron, birth weight, head circ, income **p<0.001

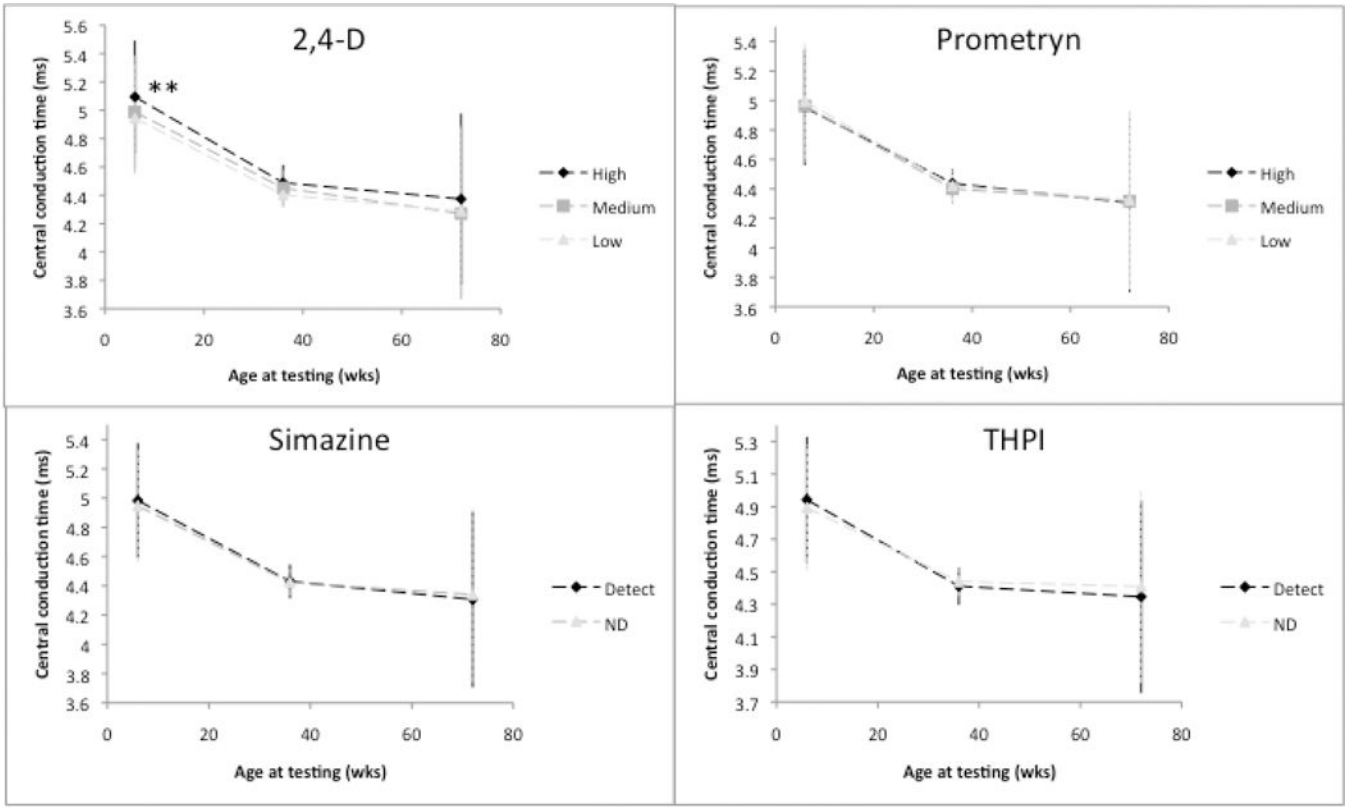

Fig 2.

Adjusted least squares means for the effect of prenatal pesticide exposure on infant ABR CCTs Mixed effects models with random intercepts; Adjusted for sex, age at testing, iron, birth weight, head circ, income **p<0.001

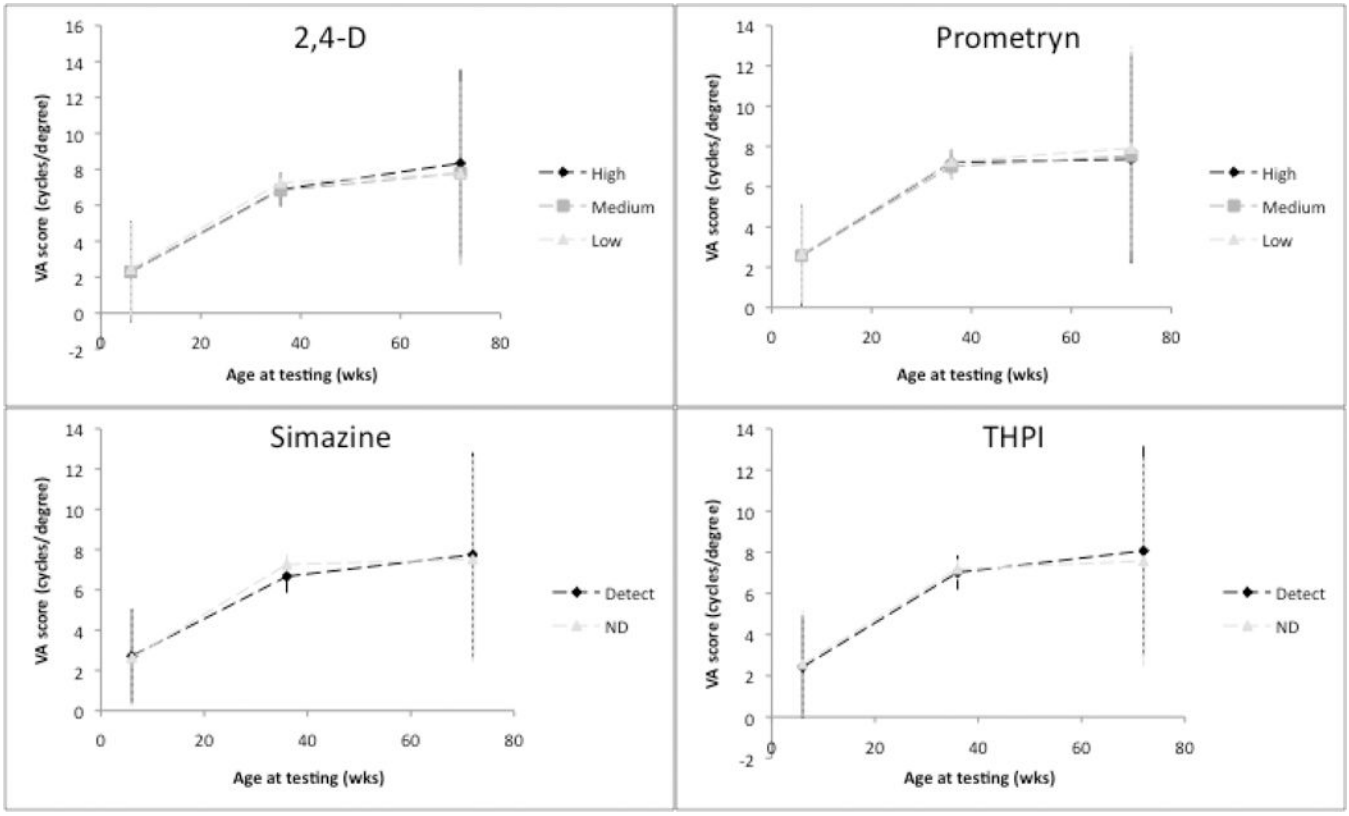

Fig 3.

Adjusted least squares means for the effect of prenatal pesticide exposure on infant grating visual acuity Mixed effects models with random intercepts; Adjusted for sex, age at testing, iron, birth weight, head circ, income

Table 1.

Distribution of fungicide and herbicide concentrations (ng/mL) in umbilical cord blood plasma samples at delivery, Zhejiang Province, China (n=232)

Pesticide	LOD	n > LOD(%)	Median	75th Pctl	90th Pctl	95th Pctl	99th Pctl	Maximum
HERBICIDES								
Atrazine	0.008	1 (0.4)	<LOD	<LOD	<LOD	<LOD	<LOD	0.01
Barban	5.57	1 (0.4)	<LOD	<LOD	<LOD	<LOD	<LOD	8.25
Dicamba	1.27	2 (0.9)	<LOD	<LOD	<LOD	<LOD	<LOD	1.87
Diphenamid	0.02	1 (0.4)	<LOD	<LOD	<LOD	<LOD	<LOD	9.09
2,4-D	0.51	63 (27.2)	<LOD	0.57	1.35	2	4.63	58.24
Diuron	0.04	1 (0.4)	<LOD	<LOD	<LOD	<LOD	<LOD	12.84
Fluridone	1.35	1 (0.4)	<LOD	<LOD	<LOD	<LOD	<LOD	10.85
Prometryn	0.02	188 (81.0)	4.37	10.88	21.65	38.26	85.81	182.18
Simazine	0.25	40 (17.2)	<LOD	<LOD	0.42	0.51	1.09	1.69
FUNGICIDES								
Dicloran	0.003	1 (0.4)	<LOD	<LOD	<LOD	<LOD	<LOD	0.05
Difenoconazole	0.07	1 (0.4)	<LOD	<LOD	<LOD	<LOD	<LOD	13.4
Dimethomorph	0.01	1 (0.4)	<LOD	<LOD	<LOD	<LOD	<LOD	10.95
Furalaxyl	0.03	1 (0.4)	<LOD	<LOD	<LOD	<LOD	<LOD	11.13
Metalaxyl	0.01	9 (3.9)	<LOD	<LOD	<LOD	<LOD	0.61	0.66
Myclobutanil	0.07	1 (0.4)	<LOD	<LOD	<LOD	<LOD	<LOD	8.52
Nuarimol	0.01	1 (0.4)	<LOD	<LOD	<LOD	<LOD	<LOD	11.9
Oxadixyl	0.01	22 (9.5)	<LOD	<LOD	<LOD	1.04	6.99	40.61
Paclobutrazole	1.35	1 (0.4)	<LOD	<LOD	<LOD	<LOD	<LOD	10.56
Triadimefon	0.07	1 (0.4)	<LOD	<LOD	<LOD	<LOD	<LOD	9.76
Triflumizole	0.02	2 (0.9)	<LOD	<LOD	<LOD	<LOD	<LOD	8.86
Quinoxyfen	0.34	2 (0.9)	<LOD	<LOD	<LOD	<LOD	<LOD	17.5
THPI	0.34	36 (15.5)	<LOD	<LOD	0.5	0.68	0.92	1.02

Table 2.

Characteristics of the study population (n=232)

Characteristics	Birth/6 weeks			9 months			18 months		
Infant	**N**	**Mean (SD)**	**Range**	**N**	**Mean (SD)**	**Range**	**N**	**Mean (SD)**	**Range**
Age at testing (days)	232	43.0 (5.1)	30–82	217	282.8(10.6)	255–314	207	554.6(10.5)	527–607
Head circ. (cm)	232	37.9 (1.2)	35.4–42.3	220	45.1 (1.4)	40.5–49.6	207	47.4(1.3)	43.6–51.5
Gestational age (wks)	207	39.0 (1.0)	37–41						
Birth weight (kg)	232	3.4 (0.4)	2.5–4.5	**N**	**N (%)**				
	N	**N (%)**							
Iron status	215			210					
Sufficient		173 (80.5)			110 (52.4)				
Deficient		42 (19.5)			100 (47.6)				
Sex	232								
Male		122 (52.6)							
Female		110 (47.4)							
Maternal/Family	**N**	**N (%)**							
Income (Yuan)	214								
<30k		43 (20.1)							
30k-49.9k		41 (19.2)							
50k-99.9k		66 (30.8)							
≥100k		64 (29.9)							
Education	220								
Middle school or less		84 (38.2)							
High/secondary school		64 (29.1)							
College		72 (32.7)							
Occupation	220								
Employed outside of home		129 (58.6)							
Housewife		91 (41.4)							

Table 3.

Adjusted linear mixed model (LMM) results for the fixed effects of prenatal pesticide exposure on infant ABR wave V latencies and CCTs

Ref group is <LOD	**ALL**		**BOYS**		**GIRLS**	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
Wave V nm	(n=232)	(n=214)	(n=122)	(n=113)	(n=110)	(n=101)
	Est. (95% Cl)	Est. (95% Cl)	Est. (95% Cl)	Est. (95% Cl)	Est. (95% Cl)	Est. (95% Cl)
2,4-D High	0.12 *	0.12 *	0.11	0.13 #	0.12 #	0.14 *
	(0.02, 0.22)	(0.03, 0.22)	(−0.05, 0.27)	(−0.03, 0.29)	(−0.00, 0.24)	(0.02, 0.26)
2,4-D Med	−0.01 (−0.12, 0.09)	−0.00 (−0.11, 0.10)	0.01 (−0.13, 0.15)	−0.01 (−0.15, 0.13)	−0.05 (−0.22, 0.12)	0.01 (−0.15, 0.17) *p-trend =0.03*
Prometryn High	−0.02 (−0.11, 0.08)	−0.01 (−0.11, 0.08)	−0.00 (−0.14, 0.13)	0.03 (−0.10, 0.17)	−0.03 (−0.17, 0.11)	−0.04 (−0.17, 0.09)
Prometryn Med	−0.01 (−0.11, 0.09)	0.02 (−0.08, 0.11)	0.01 (−0.12, 0.15)	0.08 (−0.06, 0.21)	−0.04 (−0.18, 0.10)	−0.07 (−0.20, 0.07)
Simazine Detect	0.01	0.01	−0.04	−0.04	0.05	0.07
	(−0.08, 0.10)	(−0.08, 0.10)	(−0.17, 0.10)	(−0.17, 0.10)	(−0.07, 0.17)	(−0.05, 0.18)
THPI Detect	0.02	0.02	−0.02	−0.02	0.07	0.07
	(−0.07, 0.12)	(−0.07, 0.11)	(−0.16, 0.11)	(−0.16, 0.11)	(−0.06, 0.20)	(−0.05, 0.20)
CCT nm	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
	(n=232)	(n=214)	(n=122)	(n=113)	(n=110)	(n=101)
	Estimate	Estimate	Estimate	Estimate	Estimate	Estimate
2,4-D High	0.13 **	0.15 **	0.15 #	0.16 #	0.11 #	0.14 *
	(0.04, 0.23)	(0.05, 0.25)	(−0.00, 0.30)	(−0.00, 0.32)	(−0.01, 0.24)	(−0.02, 0.27)
2,4-D Med.	0.02	0.04	0.01 (−0.12, 0.15)	0.00 (−0.14, 0.14)	0.02 (−0.15, 0.20)	0.06
	(−0.08, 0.13) *p-trend=0.009*	(−0.07, 0.14) *p-trend=0.004*			(−0.10, 0.23) *p-trend −0.02*	
Prometryn High	−0.02	−0.01	−0.01	0.04	−0.02	−0.02
	(−0.11, 0.08)	(−0.11, 0.09)	(−0.14, 0.13)	(−0.10, 0.18)	(−0.16, 0.12)	(−0.16, 0.11)
Prometryn Med	−0.02	0.01	−0.01	0.07	−0.03	−0.06
	(−0.11, 0.08)	(−0.09, 0.10)	(−0.14, 0.12)	(−0.07, 0.21)	(−0.17, 0.12)	(−0.20, 0.07)
Simazine Detect	0.03	0.03	−0.02	−0.03	0.05	0.09
	(−0.06, 0.11)	(−0.06, 0.12)	(−0.15, 0.12)	(−0.16, 0.11)	(−0.07, 0.18)	(−0.03, 0.21)
THPI Detect	0.05	0.05	0.01	−0.01	0.07	0.10
	(−0.05, 0.14)	(−0.04, 0.14)	(−0.13, 0.14)	(−0.14, 0.13)	(−0.06, 0.20)	(−0.03, 0.23)

Mixed effects models with random intercepts Model 1- adjusted for sex, age at testing, iron Model 2- adjusted for sex, age at testing, iron, birth weight, head circ, income

# p<0.10,

* p<0.05,

** p<0.001

Table 4.

Adjusted linear mixed model (LMM) results for the fixed effects of prenatal pesticide exposure on infant grating visual acuity (VA)

Ref group is <LOD	**ALL**		**BOYS**		**GIRLS**	
VA score	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
	(n=232)	(n=214)	(n=122)	(n=113)	(n=110)	(n=101)
	Est. (95% Cl)	Est. (95% Cl)	Est. (95% Cl)	Est. (95% Cl)	Est. (95% Cl)	Est. (95% Cl)
2,4-D High	−0.32	−0.41	0.24	−0.32	−0.69	−0.78
	(−1.18, 0.55)	(−1.20,0.38)	(−0.98, 1.46)	(−1.36, 0.73)	(−1.96, 0.58)	(−2.01, 0.45)
2,4-D Med.	−0.25	−0.17	−0.16	−0.03	−0.24	−0.37
	(−1.15, 0.65)	(−0.98, 0.65)	(−1.19, 0.87)	(−0.93, 0.86)	(−1.92, 1.45)	(1.91, 1.18)
Prometryn High	−0.06	−0.06	−0.27	−0.52	0.11	0.17
	(−0.91, 0.79)	(−0.83, 0.71)	(−1.32, 0.79)	(−1.44, 0.39)	(−1.31, 1.52)	(−1.14, 1.48)
Prometryn Med	−0.09	−0.33	−0.04	−0.44	−0.10	−0.05
	(−0.93, 0.76)	(−1.09, 0.43)	(−1.05, 0.98)	(−1.32, 0.44)	(−1.54, 1.35)	(−1.39, 1.29)
Simazine Detect	−0.09	−0.24	0.02	−0.21	−0.16	−0.40
	(−0.89, 0.71)	(−0.95, 0.48)	(−1.02, 1.07)	(−1.10, 0.68)	(−1.40, 1.08)	(−1.57, 0.78)
THPI Detect	−0.24	−0.36	−0.08	−0.28	−0.30	−0.51
	(−1.06, 0.59)	(−1.11, 0.38)	(−1.13,0.97)	(−1.17, 0.60)	(−1.65, 1.06)	(−1.80, 0.77)

Mixed effects models with random intercepts

Model 1 - adjusted for sex, age at testing, iron

Model 2- adjusted for sex, age at testing, iron, birth weight, head circ, income