Environment International 92–93 (2016) 478–485

Contents lists available at ScienceDirect

Environment International

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

Prenatal exposure to multiple pesticides is associated with auditory brainstem response at 9 months in a cohort study of Chinese infants

Julie Sturza [a,*], Monica K. Silver [b], Lin Xu [c], Mingyan Li [c], Xiaoqin Mai [d], Yankai Xia [e], Jie Shao [c], Betsy Lozoff [a], John Meeker [b]

[a] Center for Human Growth and Development, University of Michigan, Ann Arbor, MI, USA
[b] Department of Environmental Health Sciences, University of Michigan School of Public Health, Ann Arbor, MI, USA
[c] Department of Child Health Care, Children's Hospital Zhejiang University School of Medicine, Zhejiang, China
[d] Department of Psychology, Renmin University of China, Beijing, China
[e] State Key Laboratory of Reproductive Medicine, Institute of Toxicology, School of Public Health, Nanjing Medical University, Nanjing, China

ARTICLE INFO

Article history:
Received 12 January 2016
Received in revised form 13 April 2016
Accepted 22 April 2016
Available online xxxx

Keywords:
Pesticides
Infancy
Auditory brainstem response
Sensory function

ABSTRACT

Background: Pesticides are associated with poorer neurodevelopmental outcomes, but little is known about the effects on sensory functioning.
Methods: Auditory brainstem response (ABR) and pesticide data were available for 27 healthy, full-term 9-month-old infants participating in a larger study of early iron deficiency and neurodevelopment. Cord blood was analyzed by gas chromatography–mass spectrometry for levels of 20 common pesticides. The ABR forward-masking condition consisted of a click stimulus (masker) delivered via ear canal transducers followed by an identical stimulus delayed by 8, 16, or 64 milliseconds (ms). ABR peak latencies were evaluated as a function of masker-stimulus time interval. Shorter wave latencies reflect faster neural conduction, more mature auditory pathways, and greater degree of myelination. Linear regression models were used to evaluate associations between total number of pesticides detected and ABR outcomes. We considered an additive or synergistic effect of poor iron status by stratifying our analysis by newborn ferritin (based on median split).
Results: Infants in the sample were highly exposed to pesticides; a mean of 4.1 pesticides were detected (range 0–9). ABR Wave V latency and central conduction time (CCT) were associated with the number of pesticides detected in cord blood for the 64 ms and non-masker conditions. A similar pattern seen for CCT from the 8 ms and 16 ms conditions, although statistical significance was not reached. Increased pesticide exposure was associated with longer latency. The relation between number of pesticides detected in cord blood and CCT depended on the infant's cord blood ferritin level. Specifically, the relation was present in the lower cord blood ferritin group but not the higher cord blood ferritin group.
Conclusions: ABR processing was slower in infants with greater prenatal pesticide exposure, indicating impaired neuromaturation. Infants with lower cord blood ferritin appeared to be more sensitive to the effects of prenatal pesticide exposure on ABR latency delay, suggesting an additive or multiplicative effect.

1. Introduction

Billions of pounds of synthetic pesticides are applied globally each year for crop protection and pest management in agricultural and residential settings (Burns et al., 2013). The highest usage occurs in the agricultural sector, with over 4.6 million tons applied each year (Zhang et al., 2011; EPA, 2011). China is one of the world's largest consumers of agricultural pesticides (Zhang et al., 2011; EPA, 2011; Ding and Bao, 2013), applying 2.5–5 fold more per field unit than the global average (Zhang et al., 2014). In Zhejiang province, the site of this study, agricultural applications are some of the highest in China, at nearly double the national rate (Huang et al., 2001). Due to their heavy use in agriculture, non-occupational pesticide exposure is most likely to occur via consumption of contaminated food. Additional exposure may also occur via contaminated drinking water, dust, and spray drift, especially in rural, farming communities, as well as from the use of residential pesticides in the home or yard (Huang et al., 2001).

Synthetic pesticides are toxic to biological systems by design. Many act by disrupting signaling mechanisms in the central nervous system (CNS) thereby inhibiting neurological function. Evidence from animal studies and adult occupational poisonings has demonstrated that

* Corresponding author at: Center for Human Growth and Development, University of Michigan, 300 North Ingalls Street 1066 NE, Ann Arbor 48109-0406, MI, USA.
E-mail addresses: jmigrin@umich.edu (J. Sturza), mksilver@umich.edu (M.K. Silver), xlin33@163.com (L. Xu), li.mingyan1984@gmail.com (M. Li), maixq@ruc.edu.cn (X. Mai), yankaixia@njmu.edu.cn (Y. Xia), shaojie@zju.edu.cn (J. Shao), blozoff@umich.edu (B. Lozoff), meekerj@umich.edu (J. Meeker).

http://dx.doi.org/10.1016/j.envint.2016.04.035
0160-4120/

these insecticides act via similar neurotoxic mechanisms in mammals following high-dose exposure (Abdollahi and Karami-Mohajeri, 2012; Yang and Deng, 2007). Less is known about the mechanisms of neurotoxicity at low-level exposures that are relevant to the general population. Low-level pesticide exposures are an important concern in pregnant women and young children. Fetal and infant brains are rapidly developing, leaving them highly vulnerable to potentially long-lasting effects of pesticide exposure, such as disruption of brain architecture or circuitry (Garcia et al., 2005). Adding to concerns for fetal exposure, pesticides are able to cross the placenta (Bradman et al., 2003) and fetuses tend to have lower levels of detoxifying enzymes (Eskenazi et al., 2008), both of which are thought to increase fetal susceptibility. Low-level pesticide exposures during pregnancy or childhood have been found to be associated with neurodevelopmental deficits such as lower IQ (Bouchard et al., 2011; Rauh et al., 2011; Eskenazi et al., 2007; Engel et al., 2011) and disorders such as autism (Roberts et al., 2007; Shelton et al., 2014), attention deficit-hyperactivity disorder (Marks et al., 2010; Bouchard et al., 2010; Rauh et al., 2006), and pervasive developmental disorder (Eskenazi et al., 2007; Rauh et al., 2006).

Very little is known about the effects of early-life pesticide exposure on the auditory pathways of the brain or other sensory systems. There have been some reports of hearing loss and ototoxicity following pesticide exposure, but most of the evidence comes from animal or occupational case studies where high-level exposures are the norm (Gatto et al., 2014). One recent study found deficits in cochlear status in children exposed to organochlorine pesticides (Sisto et al., 2015). Regarding visual sensory function, a recent study in an Arctic population with high DDE exposure found that both pre- and postnatal DDE exposure were associated with visual processing impairment at school age (Cartier et al., 2014).

Auditory system development in infancy helps to provide the foundation for subsequent communication and language acquisition in childhood (Chonchaiya et al., 2013; Algarin et al., 2003). Therefore, early life deficits in auditory function, potentially as a result of prenatal pesticide exposure, could contribute to detrimental long-term effects on learning or other cognitive functions later in childhood (Chonchaiya et al., 2013; Molfese, 1989; Molfese, 2000; Benasich and Tallal, 2002).

Nutrient-toxicant interactions are an important area of research given that concurrent exposure to toxicants and early-life nutrient deficiencies are common in many parts of the world. Iron is an important nutrient for early neurodevelopment (Lozoff, 2007; Georgieff et al., 2015) and an essential factor in myelination and oligodendrocyte biology (Badaracco et al., 2010; Todorich et al., 2009). Previous studies that found longer ABR latencies among iron-deficient infants are consistent with impaired myelination of the auditory pathways (Algarin et al., 2003; Roncagliolo et al., 1998). Because iron and pesticides both appear to have impacts on neurodevelopment and myelination, it is relevant to consider them jointly, specifically in regard to auditory system development.

The principle aim of this pilot study was to explore the effects of environmental exposures to multiple pesticides on infant auditory function at nine months, as measured by auditory brainstem response (ABR). A secondary aim was to explore the pesticide-iron interaction as it relates to ABR in infants.

2. Materials and methods

2.1. Ethics statement

Signed written informed consent was obtained from infants' parents. All study protocols were approved by the Institutional Review Boards and ethics committees at both the University of Michigan and Children's Hospital of Zhejiang University.

2.2. Participants

The study population was a subset of Chinese infants participating in a study of early iron deficiency and neurodevelopment jointly conducted by the University of Michigan and the Children's Hospital of Zhejiang University. Women/infant pairs meeting the following criteria were invited to participate: singleton full-term birth (37–41 weeks completed gestation); birth weight > 2500 g; no perinatal complications or congenital malformations; no general undernutrition (<10th percentile for weight or length); no acute or chronic illness; no multiple or prolonged hospitalizations (>5 days). Consecutive participants in the study with due dates between April and June 2009 ($n = 116$) were included in this pilot study of pesticide exposure; 27 of the infants had ABR data.

2.3. Cord blood pesticides

Cord blood collection and pesticide analysis methods have been described elsewhere (Wickerham et al., 2012). Briefly, a 30 mL umbilical cord blood sample was collected, separated, and serum was stored at −80 °C. Serum samples underwent solid-phase extraction and analysis using isotope dilution gas chromatography–mass spectrometry (GC–MS) at Nanjing Medical University in Nanjing, China. Pesticides were selected based on usage data, availability of analytical standards, method compatibility, and preliminary data. The final list included: organophosphate insecticides (chlorpyrifos, diazionon, fonofos, malathion, parathion-ethyl, parathion-methyl, profenofos, terbufos), carbamate insecticides (carbofuranphenol, propoxur), herbicides (acetochlor, alachlor, atrazine, linuron, metolachlor, trifluralin), fungicides (dicloran, metalaxyl, vinclozolin), and repellant (diethyltoluamide). Pesticide concentrations were analyzed by Thermo Trace GC and DSQ Mass Spectrometer (Thermo, USA). Limits of detection (LOD) ranged from 0.05 ng/mL to 0.50 ng/mL (Table 1). Quality control samples were analyzed in parallel with unknown samples in each analytical series.

Table 1
Distribution of pesticide concentrations in umbilical cord serum (ng/ml) at delivery, Zhejiang Province, China 2009 ($n = 27$).

Pesticide	Limit of detection (LOD) (ng/ml)	Number of infants with levels > LOD (%)	Selected percentiles				
			50	75	90	95	max
Organophosphates							
Chlorpyrifos	0.05	5 (19%)	ND	ND	0.16	0.17	0.17
Diazinon	0.05	2 (7%)	ND	ND	ND	0.45	0.87
Fonofos	0.05	3 (11%)	ND	ND	0.26	0.56	1.06
Malathion	0.50	5 (19%)	ND	ND	2.87	2.88	3.06
Parathion-ethyl	0.05	0 (0%)	ND	ND	ND	ND	ND
Parathion-methyl	0.05	6 (22%)	ND	ND	1.83	2.14	2.53
Profenofos	0.50	10 (37%)	ND	0.68	0.74	0.84	0.96
Terbufos	0.05	7 (26%)	ND	0.14	0.34	0.39	0.39
Carbamates							
Carbofuranphenol	0.05	9 (33%)	ND	20.60	31.22	32.34	46.10
Propoxur	0.05	0 (0%)	ND	ND	ND	ND	ND
Herbicides							
Acetochlor	0.50	5 (19%)	ND	ND	0.61	0.82	0.99
Alachlor	0.05	8 (30%)	ND	0.08	3.26	3.82	5.26
Atrazine	0.25	7 (26%)	ND	0.67	1.53	1.68	1.83
Linuron	0.50	1 (4%)	ND	ND	ND	ND	1.10
Metolachlor	0.05	0 (0%)	ND	ND	ND	ND	ND
Trifluralin	0.05	0 (0%)	ND	ND	ND	ND	ND
Fungicides							
Dicloran	0.05	8 (30%)	ND	1.22	7.87	9.06	11.46
Metalaxyl	0.05	0 (0%)	ND	ND	ND	ND	ND
Vinclozolin	0.05	16 (59%)	0.36	0.60	1.31	1.61	1.85
Repellent							
Diethyltoluamide	0.05	20 (74%)	0.29	0.52	0.78	0.98	1.08

ND = non-detectable.

Binary exposure variables (≥LOD = 1, <LOD = 0) were created for each of the pesticides of interest and summed to determine the total number of pesticides detected in cord blood; this was our primary independent variable. In this sense, we hoped to estimate the additive or synergistic impact of exposure to multiple pesticides (Rider et al., 2010; Wickerham et al., 2012). We also calculated the total number of detected pesticides in the following four categories: organophosphates, carbamates, herbicides, and fungicides. Given the small sample size of this pilot study and the relatively high LODs for the pesticides, we felt this method of exposure classification was most appropriate.

2.4. Auditory brainstem response (ABR) recording

ABR is a highly reliable, noninvasive way of measuring the maturation of the auditory pathway, from the periphery to the brainstem, as well as overall central nervous system maturation in infants (Despland and Galambos, 1980; Jacobson, 1985; Song et al., 2011). A series of clicks are transmitted via ear canal transducers, and electroencephalogram (EEG) electrodes on the scalp record the neural activity at auditory centers along the brainstem. ABRs typically consist of three well-defined peaks in infants, corresponding to nerve activation in the cochlear nerve (wave I), the cochlear nuclei (wave III), and the lateral lemniscus (wave V) (Hall, 2007; DeBonis and Donohue, 2008). ABR peak latencies decline throughout infancy, indicating faster signal transmission, as the auditory pathways mature and become increasingly myelinated.

For this study, ABRs were obtained from nine-month-old infants during unsedated sleep following an initial test for basic hearing function (Despland and Galambos, 1980). ABR testing was carried out using a Biologic Navigator (Bio-Logic Systems Corp., Mundeleiu, IL)/Traveler evoked potential system. We assessed temporal processing abilities (rapid acoustic processing) by using a forward masking paradigm (Mai et al., 2015). The use of the forward-masking paradigm in this sample is described in detail elsewhere (Chonchaiya et al., 2013). Briefly, the forward-masking paradigm has been used to investigate the temporal processing and frequency discrimination of the auditory system (Chonchaiya et al., 2013; Lasky, 1991; Walton et al., 1999). It differs from standard ABR because as long as the signal and the masker are closely spaced (70 ms or less), both signals activate auditory nerve fibers, and nerve fibers responding to the first stimulus have not recovered to become available to respond to the second stimulus (Chonchaiya et al., 2013). The closer the masker is to the stimulus, the longer it takes for the auditory nerve fibers to recover and become available to respond (Abbas and Gorga, 1981).

ABR recordings used clicks presented at 80 decibels above normal adult hearing level (dB nHL) (the signal), which were preceded by another click (the masker) at different time intervals: 8, 16, and 64 milliseconds (ms). Each averaged response consisted of 1300 accepted sweeps, which was then replicated, yielding a waveform representing 2600 responses. The data acquisition program automatically rejected any traces contaminated by high-amplitude artifacts (voltage exceeding ±23.80 μV). The latency and amplitude values obtained for the right and left ears were averaged. ABR waveforms were analyzed for latencies for component peaks I, III, and V. We focused on wave V latency and central conduction time (CCT), which is the interpeak latency from wave I to wave V. These measures have been suggested as useful measures of auditory processing because they are easily identifiable and reproducible (Berglund et al., 2011). Wave V is frequently used as an indicator of the neurological integrity of the auditory system (Hecox and Galambos, 1974).

2.5. Statistical analysis

Data analysis was conducted with SAS 9.4 (SAS Institute Inc., Cary, NC). Descriptive statistics, frequencies, and correlations were examined for variables of interest. To address our primary research question, linear regression models were used to evaluate associations between total number of all pesticides detected and ABR outcomes (wave V latencies and CCT at the 8, 16, and 64 ms conditions, as well as the non-masker condition) at nine months of age. As a secondary analysis, we then repeated these models while replacing total number of detected pesticides with total number of detected organophosphates, carbamates, herbicides, or fungicides. To examine the dose-response relationship of pesticides which were detected in >50% of the samples, we used linear regression models where vinclozolin and diethyltoluamide predicted ABR outcomes. Samples that fell below the LOD were imputed as LOD divided by the square root of two. In addition to the main effects of the primary pesticide exposure variable, we considered an additive or synergistic effect of poor iron status by stratifying our analysis by newborn ferritin (based on median split of 146 ng/mL).

We examined bivariate associations between potential covariates and the various ABR outcomes of interest using t-tests and Pearson correlation coefficients, as well as the bivariate associations between potential covariates and our pesticide exposure index variable. The small sample size with ABR data in this pilot study limited power to include covariates in the pesticide-iron interaction analysis. Small sample size also makes results sensitive to the impact of outliers. One child had ABR CCT and wave V latency values >2.5 standard deviation units from the mean. These values were removed before final analysis.

3. Results

The mean number of pesticides detected was 4.1, with a standard deviation of 2.3 and a range of 0–9. Only one infant had no pesticides detected in cord blood. A full description of the sample is shown in Tables 1 and 2. Child sex was the only background variable that showed consistent statistically significant associations with the various ABR wave V latencies and CCTs. However, child sex was not statistically significantly associated with the total number of detected pesticides, eliminating concern about potential confounding. We therefore did not include child sex as a covariate in our final models.

Given the small sample size, we closely examined the distributions of the dependent variables before pursuing linear regression to avoid violating model assumptions. We used SAS Proc Univariate to display histograms to allow for visual inspection of data distributions, as well as to perform tests for normality (Shapiro–Wilk, Kolmogorov–Smirnov, Cramer–von Mises, Anderson–Darling). All tests indicated that all dependent variables were normally distributed. Based on linear regression models of ABR wave V latencies and CCT (Table 3), the total number of pesticides detected was significantly associated with the wave V latency for the 64 ms condition ($p = 0.03$) and approached statistical significance for the non-masker condition ($p = 0.05$). Interpeak latencies from wave I to V (CCT) were significantly associated with the total

Table 2
Characteristics of the study population.

Variable	Sample with ABR data ($n = 27$)
Child male, n (%)	15 (55.6%)
Total number of pesticides detected (M and SD)	4.1 (2.3)
Total number of organophosphate pesticides detected (M and SD)	1.48 (1.16)
Total number of carbamate pesticides detected (M and SD)	0.33 (0.48)
Total number of herbicides detected (M and SD)	0.74 (0.65)
Total number of fungicides detected (M and SD)	0.96 (0.76)
Birth weight (grams, M and SD)	3393 (485)
Gestational age (weeks, M and SD)	39.4 (1.2)
Cord blood ferritin (ng/mL)	162.9 (81.1)
Weight-for-age *z*-score at 9 month ABR testing (M and SD)	0.73 (0.93)
Age at 9 month ABR testing (months, M and SD)	9.6 (0.5)
Head circumference-for-age *z*-score at 9 month ABR testing (M and SD)	0.08 (0.95)
Father smokes, n (%)	15 (55.6%)
Cord blood lead (μg/dL, M and SD)	3.3 (1.3)

Table 3
Parameter estimates for models predicting ABR Wave V and CCT from total number of detected pesticides and number of pesticides detected in each category.

	Non-masker		8 ms masker		16 ms masker		64 ms masker	
	Wave V	CCT	Wave V	CCT	Wave V	CCT	Wave V	CCT
	$N = 27$	$N = 27$	$N = 20$	$N = 19$	$N = 21$	$N = 21$	$N = 27$	$N = 26$
Total pesticides detected (95% CI)	0.03 (0.00–0.06)***	0.04 (0.01–0.07)*	0.02 (−0.02–0.05)	0.03 (0.00–0.07)***	0.02 (−0.02–0.07)	0.03 (−0.01–0.08)†	0.04 (0.00–0.08)*	0.04 (0.01–0.07)**
Organophosphates	0.04 (−0.03–0.11)	0.05 (−0.01–0.12)	0.01 (−0.06–0.08)	0.05 (−0.02–0.13)	0.03 (−0.06–0.12)	0.03 (−0.06–0.12)	0.04 (−0.04–0.12)	0.05 (−0.02–0.12)
Carbamates	0.09 (−0.08–0.25)	0.05 (−0.13–0.23)	0.02 (−0.16–0.20)	0.00 (−0.20–0.20)	0.07 (−0.15–0.28)	0.09 (−0.13–0.31)	0.20 (0.02–0.38)*	0.09 (−0.08–0.26)
Herbicides	0.07 (−0.04–0.18)	0.10 (−0.02–0.21)***	−0.01 (−0.14–0.13)	0.06 (−0.08–0.19)	0.01 (−0.14–0.17)	0.05 (−0.11–0.21)	0.09 (−0.05–0.23)	0.11 (−0.01–0.22)***
Fungicides	0.07 (−0.02–0.17)	0.08 (−0.02–0.18)†	0.06 (−0.05–0.18)	0.07 (−0.05–0.19)	0.06 (−0.08–0.21)	0.09 (−0.05–0.24)	0.11 (−0.01–0.22)†	0.11 (0.01–0.20)*

Ns vary based on availability of useable ABR data.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.10$.

number of detected pesticides for the 64 ms and non-masker condition ($p = 0.008$ and $p = 0.01$, respectively). This pattern was also seen for the 8 ms and 16 ms conditions, although statistical significance was not reached ($p = 0.06$ and $p = 0.09$, respectively). In all models, increased pesticide exposure was associated with longer latency.

The small sample size was a concern, especially for the 8 ms and 16 ms condition where data availability limited the analytic sample more than it did for the 64 ms and non-masker condition. In our primary models where total detected pesticides predicted CCT, the beta coefficients were similar in magnitude for all four conditions, yet only significant for the non-masker and 64 ms conditions (Table 3, first row). Statistical power to detect the differences found in the 8 ms and 16 ms condition models was 0.35 and 0.25, respectively, leading us to believe that the lack of significant findings in these models may be due, at least partially, to the smaller sample size rather than a lack of a true association.

The results for the secondary models with number of detects in the four pesticide classes (Table 3) showed only two significant findings; the number of detected carbamates predicted longer wave V latency on the 64 ms condition and the number of fungicides detected predicted longer CCT on the 64 ms condition ($p = 0.03$ for both models).

Results of the dose-response analyses for vinclozolin and diethyltoluamide are shown in Table 4. There was a statistically significant linear association between vinclozolin concentration and wave V and CCT from the 8 ms condition. There was a statistically significant association between diethyltoluamide and wave V and CCT for the 16 ms and 64 ms conditions. In all cases, a higher cord blood concentration of the pesticide was associated with longer latencies providing evidence of a linear dose-response relation.

In our secondary subgroup analyses, the relation between number of pesticides detected in cord blood and CCT depended on the infant's cord blood ferritin level (Figs. 1 and 2). Specifically, the relation was present in the lower cord blood ferritin group ($p = 0.009$ for non-masker condition; $p = 0.01$ for 64 ms condition) but not the higher cord blood ferritin group ($p = 0.34$ for non-masker condition; $p = 0.26$ for 64 ms condition).

4. Discussion

This study demonstrated an association between prenatal exposure to multiple pesticides (as measured in cord blood) and infant auditory function at 9 months of age. Infants who were exposed to higher numbers of pesticides prenatally had longer wave 5 latencies and CCT intervals on ABR testing. These associations seemed to be strongest in infants with lower levels of cord ferritin. The fact that the findings were strongest for the 64 ms forward-masking condition was important given that performance on this condition was associated with language acquisition at 9 months in the same sample (Chonchaiya et al., 2013).

Testing the association between ABR function and each pesticide class individually showed limited statistically significant results, but echoed the findings of the total pesticide models with trends toward longer latencies with more pesticide exposure and significant results concentrated in the 64 ms condition. It did not allow us to pinpoint which specific class or classes are most important when considering the link between prenatal pesticide exposure and auditory processing in infancy. Dose-response models for vinclozolin and diethyltoluamide suggested that ABR latencies increase linearly with increasing cord blood concentrations, at least for these highly-detected pesticides.

To our knowledge, this is only the second study to examine pesticide exposures occurring prenatally and auditory function in human infants (Sisto et al., 2015). Considering how a nutritional deficiency, such as limited iron stores, might affect relations between pesticide exposure and infant auditory function is another novel aspect of this pilot study.

Occupational and laboratory studies provide some limited evidence of hearing loss and deficits in auditory-related function and morphology following high exposures to some pesticides (Gatto et al., 2014). Studies

Table 4
Parameter estimates for models predicting ABR Wave V and CCT from vinclozolin and diethyltoluamide concentrations.

	Non-masker		8 ms masker		16 ms masker		64 ms masker	
	Wave V	CCT	Wave V	CCT	Wave V	CCT	Wave V	CCT
	$N = 27$	$N = 27$	$N = 20$	$N = 19$	$N = 21$	$N = 21$	$N = 27$	$N = 26$
Vinclozolin (ng/mL)	0.14 (−0.01–0.28)***	0.12 (−0.03–0.28)	0.20 (0.05–0.36)*	0.18 (0.01–0.35)*	0.14 (−0.07–0.36)	0.12 (−0.10–0.35)	0.10 (−0.08–0.29)	0.15 (0.00–0.30)***
Diethyltoluamide (ng/mL)	0.23 (−0.01–0.48)***	0.24 (−0.02–0.49)***	0.24 (0.00–0.48)***	0.21 (−0.05–0.48)	0.35 (0.04–0.67)*	0.42 (0.11–0.73)**	0.34 (0.04–0.63)*	0.26 (0.01–0.51)*

Ns vary based on availability of useable ABR data.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.10$.

of farm workers, agricultural crop sprayers, and insecticide sprayers for mosquito control programs have reported hearing loss/poorer scores on audiometric tests (Beckett et al., 2000; Guida et al., 2010; Hoshino et al., 2008; Harell et al., 1978), hearing dysfunction (Teixeira et al., 2003), and self-reported reduced hearing capacity (Crawford et al., 2008). Relationships between pesticide exposure and hearing loss in occupational settings may be confounded by co-exposure to noise, however, making it difficult to study the effects of pesticides alone (Gatto et al., 2014). Laboratory studies using animals have also reported evidence of hearing loss (Hadjab et al., 2004), increased thresholds (Hadjab et al., 2004; Bergler et al., 1996), increased ABR inter-peak latencies (Bergler et al., 1996), altered cochlear morphology (Korbes et al., 2010), and decreased numbers of outer and inner hair cells (Bielefeld et al., 2005; Nicotera et al., 2004), though results vary largely depending on type of pesticide, and route and duration of exposure.

The mechanism of ototoxicity following high levels of pesticide exposure is believed to target the sensitive outer hair cells of the corti organ in the inner ear (Gatto et al., 2014). Free radical and reactive oxygen species generation are thought to lead to increased apoptosis in this region of the ear because of its low levels of glutathione peroxidase, making it more vulnerable to the effects of oxidative stress (Cardinaal et al., 2000). The mechanism for low-dose developmental toxicity is less clear. However, a recent study of environmental exposure to organochlorine pesticides in children found evidence of deficits in cochlear status (Sisto et al., 2015).

One potential mechanism for the developmental neurotoxicity of low-dose pesticide exposure is the disruption of oligodendrocyte development and function in the brain. Several laboratory studies show that oligodendrocytes may actually be more sensitive to low-level pesticide exposure than neurons (Garcia et al., 2001; Garcia et al., 2002; Garcia et al., 2003). Oligodendrocytes are responsible for the synthesis and maintenance of the myelin sheaths that surround neuronal axons. Myelin insulates, protects, and enhances the speed and quality of the transmission of action potentials that move along neurons (Tau and Peterson, 2010). Premyelinating oligodendrocytes populate the developing cortex during gestation and may be particularly sensitive to the effects of prenatal pesticide exposure. Disruption of oligodendrocytes early in life could potentially lead to deficits in myelination and predispose infants to poorer cognitive and neurodevelopmental outcomes later in childhood (Tau and Peterson, 2010). Myelination of the human brain begins in the third trimester, with sensory tracts being myelinated first (Tau and Peterson, 2010; Carlson, 2014).

Previous research on the effects of pesticide exposure on oligodendrocyte function and brain myelination has been in rats and has focused largely or primarily on organophosphate (OP) insecticides, specifically chlorpyrifos (CPF) and diazinon (DZN), or the broad-leaf herbicide, 2,4-dichlorophenoxyacetic acid (2,4-D). Pups born to pregnant rats treated with CPF during gestation or postnatally, corresponding to the onset of myelination in rats, showed immediate and long-term deficits in an oligodendrocyte-specific marker, myelin basic protein (MBP) (Garcia et al., 2002; Garcia et al., 2003). Another study exposed neonatal rats to CPF or DZN and found that CPF evoked a decrease in overall expression of myelin-related genes, as well as eliciting statistically significant deficits in the expression of specific myelin-related genes. Treatment with DZN did not have an overall effect on myelin gene expression, but elicited a statistically significant reduction in individual myelin-related gene expression (Slotkin and Seidler, 2007). Similarly, neonatal exposure to 2,4-D resulted in myelin deficits in the rat pup brain, as measured by protein expression (Duffard et al., 1996; Konjuh et al., 2008) and electron microscopy (Konjuh et al., 2008; Rosso et al., 2000), and alterations in behavior (Rosso et al., 2000).

Taken together, these studies indicate that exposure to CPF, DZN, or 2,4-D during the developmental period corresponding to the onset of myelination can induce negative effects on myelin-related gene expression and function and myelination in the brain. Though much of the work to this point has focused on a few select pesticides, the results

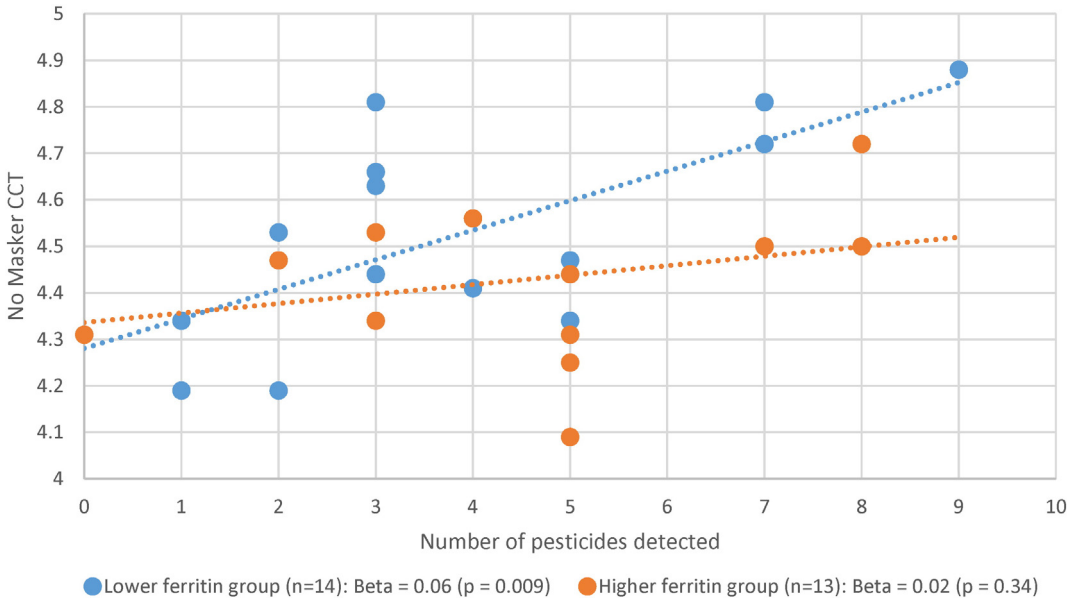

Fig. 1. Pesticide–CCT relation by newborn ferritin group, non-masker condition.

may be pertinent to other pesticides. Despite their differing modes of action at high doses, these different classes of pesticides appear to elicit similar effects on myelination in in vivo laboratory studies.

While we were unable to examine the effects of CPF or DZN alone due to low levels of detection, our ABR results support the hypothesis that pesticide exposure inhibits myelination. Here we observed that exposure to a higher number of pesticides during pregnancy was associated with longer CCT and wave V latencies. Longer CCT intervals and wave V latencies indicate slower auditory transmission speeds. Since myelination occurs centripetally, from the more central to the more distal parts of the pathway, the wave V latency is expected to increase with a disorder of myelination (Jiang, 1995). Additionally, myelination of the auditory pathways begins late in gestation in the human. Thus, pesticide levels in umbilical cord blood may adequately reflect fetal exposure during the onset of myelination.

We chose to examine a nutrient-toxicant interaction, the pesticide-iron effect, because iron is important for early neurodevelopment and concurrent exposure to pesticides and early-life iron deficiency is common in many parts of the world. Iron is essential for oligodendrocyte function and myelination (Badaracco et al., 2010; Todorich et al., 2009). Iron deficiency alters myelin-related genes and proteins in the short- and long-term in developing rats and primate infants (Beard and Connor, 2003; Beard, 2007; Siddappa et al., 2003; Clardy et al., 2006). Previous findings of longer ABR latencies in iron-deficient human infants are consistent with impaired myelination of the auditory pathways (Algarin et al., 2003; Roncagliolo et al., 1998). Our subanalyses of the pesticide effect in infants with lower vs. higher ferritin at birth are consistent with these previous findings. We observed slower auditory transmission (longer CCTs) in infants with more pesticide exposures and lower cord blood ferritin levels compared with similarly

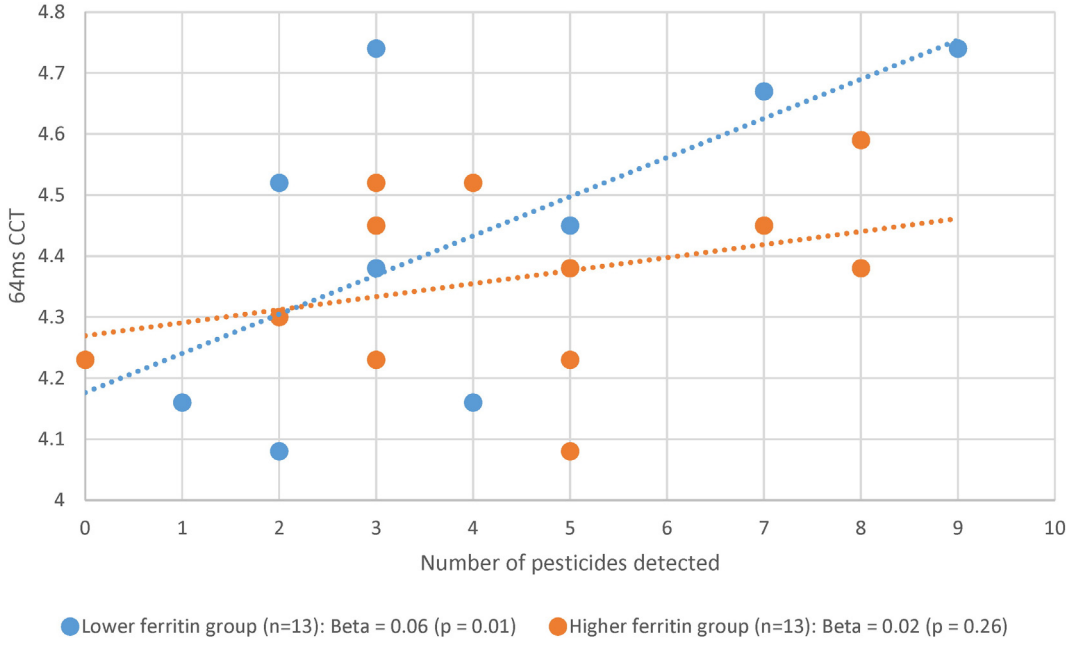

Fig. 2. Pesticide–CCT relation by newborn ferritin group, 64 ms condition.

exposed infants with higher ferritin levels at birth, suggesting that there may be important ferritin-pesticide interactions.

Our small sample size limits both our statistical power for detecting subtle associations and our ability to adjust for possible confounding variables. The study is further restricted by the methods of pesticide detection. The relatively high LODs limited our ability to assess relations between individual pesticides or mixtures and ABR outcomes more quantitatively. Exposure measurement at only one time point (delivery) means that we cannot characterize pesticide exposure throughout pregnancy and may miss other potentially sensitive windows of exposure. Finally, the results may not adequately account for the effects of other pesticides that may be present, since only a selection of non-persistent pesticides were measured in cord blood. We anticipate that many of these concerns will be ameliorated when this pilot study is expanded to a larger sample.

Despite its limitations, this is only the second study to examine the effects of prenatal pesticide exposures and human auditory function. By examining a relatively large number and variety of pesticides we could begin to explore the effects of the multiple exposures, unlike many other studies that focus on only one pesticide or metabolite. Also, measuring pesticide levels in cord blood provided stronger evidence of exposure than non-specific urinary metabolites (Barr et al., 1999; Munoz-Quezada et al., 2013). Finally, the use of ABR to assess of auditory function provides a noninvasive indication of infant auditory system myelination, giving insight into a possible mechanism for low-level pesticide neurotoxicity.

5. Conclusions

This work provides preliminary evidence that auditory system maturation may be delayed in infants with multiple prenatal pesticide exposures and that this effect is strongest in infants with lower iron stores at birth. The auditory system starts myelinating in late gestation and matures rapidly in infancy. Longer CCT and wave V latencies suggest that exposure to multiple pesticides prenatally has negative effects on auditory system myelination early in life. Auditory system development in infancy provides the foundation for many subsequent learning processes, such as communication and language development (Chonchaiya et al., 2013; Algarin et al., 2003). Therefore, delays or altered timing of auditory systems myelination, related to prenatal pesticide exposures, may contribute to detrimental long-term effects on learning or other cognitive functions in childhood and possibly beyond. Larger studies of pesticide exposure, iron deficiency, and auditory system development are needed to verify the findings of this pilot study.

List of abbreviations

ABR	auditory brainstem response
CNS	central nervous system
GC–MS	gas chromatography–mass spectrometry
LOD	limit of detection
EEG	electroencephalogram
dB nHL	decibel above normal adult hearing level
ms	milliseconds
CCT	central conduction time
ng/mL	nanogram per milliliter
μV	microvolt
SD	standard deviation
SE	standard error
ND	non-detect
OP	organophosphate
CPF	chlorpyrifos
DZN	diazinon
2,4-D	2,4-dichlorophenoxyacetic acid

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

JSturza drafted and revised the paper, conducted data analysis, and approved the final manuscript as submitted.

MKS drafted and revised the paper, and approved the final manuscript as submitted.

LX collected data, critically revised the manuscript, and approved the final manuscript as submitted.

ML collected data, critically revised the manuscript, and approved the final manuscript as submitted.

XM processed ABR data, critically revised the manuscript, and approved the final manuscript as submitted.

YX processed cord blood samples, critically revised the manuscript, and approved the final manuscript as submitted.

JShao contributed to study conception and design, conducted the research, critically revised the manuscript, and approved the final manuscript as submitted.

BL conceptualized and designed the study, interpreted data, critically revised the paper, and approved the final manuscript as submitted.

JM conceptualized and designed the study, interpreted data, critically revised the paper, and approved the final manuscript as submitted.

Acknowledgments

We are grateful to the families who participated in the study, the nurses and colleagues at Children's Hospital of Zhejiang University School of Medicine for their dedicated assistance, and Paul R. Kileny, University of Michigan, for help with training in ABR data recording and processing. This study was supported by grants from the National Natural Science Foundation of China (#81273085), the US National Institutes of Health (P01 HD039386), the US National Institute for Environmental Health Sciences (R01 ES021465), and the Michigan Institute for Clinical and Health Research (UL 1RR024986).

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