Prenatal Pesticide Exposure and Infant Neurodevelopment in China

by

Monica Karol Silver

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Environmental Health Sciences)
in the University of Michigan
2017

Doctoral Committee:

Professor John D. Meeker, Chair
Research Scientist Niko Kaciroti
Professor Betsy Lozoff
Associate Professor Marie S. O'Neill
Professor Peter X.K. Song

Monica Karol Silver

mksilver@umich.edu

ORCID iD: 0000-0002-5920-6906

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. John Meeker, for his expertise, patience, and good humor in guiding me through the doctoral program. John afforded me every opportunity to be successful and I am so thankful for his absolute support. Also, thank you to my committee members, Dr. Betsy Lozoff, Dr. Niko Kaciroti, Dr. Marie O'Neill, and Dr. Peter Song, for their enthusiasm, insightful comments, and expertise. Special thanks to Betsy and Niko for all of the time they spent teaching me about this cohort and its complex neurodevelopmental outcomes. I would additionally like to acknowledge my funding sources: the University of Michigan Rackham Merit Fellowship, the Ruth L. Kirschstein National Research Service Award from University of Michigan's NIOSH-ERC, and grant R01ES021465 from NIEHS. I would also like to thank my wonderful friends and colleagues in the Environmental Health Sciences department and everyone in the Meeker research group. I'm not sure I could have survived the PhD program without you! Finally, I am forever grateful to my parents, Ed and Kathy Silver, for all of their love, guidance, and support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ABR	Auditory brainstem response
AChE	Acetylcholinesterase
ADHD	Attention-deficit/hyperactivity disorder
ASD	Autism spectrum disorder
CARB	Carbamate
CI	Confidence internal
CNS	Central nervous system
DDT	Dichloro-diphenyl-trichloroethane
FM	Fine motor raw score
FMQ	Fine motor quotient
GM	Gross motor raw score
GMQ	Gross motor quotient
HC	Head circumference
INFANIB	Infant neurological international battery
IPI	Inter-peak interval
LBW	Low birth weight
LOD	Limit of detection
MRI	Magnetic resonance imaging
MST	Medial superior temporal gyrus
MT	Middle temporal gyrus
ND	Non-detect
OC	Organochlorine
OP	Organophosphate
PDMS-2	Peabody developmental motor scales, 2nd edition
PYR	Pyrethroid
SD	Standard deviation
TM	Total motor raw score
TMQ	Total motor quotient
VA	Visual acuity
V-M	Visual-motor integration
5HT	Serotonin

ABSTRACT

Over 15% of children worldwide suffer from neurodevelopmental disorders and diagnoses of autism spectrum disorder and attention-deficit/hyperactivity disorder have been increasing over the past several decades. Widespread use of synthetic pesticides has concurrently grown, raising concerns that pesticide exposure may be contributing to the rise in prevalence of these disorders. Synthetic pesticides are toxic to biological systems by design, with neurotoxicity and disruption of central nervous system signaling as the primary mode of action for many. Early-life pesticide exposures during periods of rapid development are of particular concern for neurodevelopment because of the potential for long-term effects. China is the world's largest consumer of pesticides, but despite a potentially highly exposed population, very little is known about the levels of prenatal pesticide exposure or predictors of those exposures in China.

Organophosphate insecticides (OPs) are used worldwide, and account for more than a third of all pesticide use in China, yet despite nearly ubiquitous exposure in the general population, few have been studied for neurodevelopmental effects. Laboratory studies indicate that OPs negatively affect a host of neuronal processes and epidemiological studies report associations between prenatal OP exposure and increased prevalence of

neurological disorders in childhood. Little is known about how OP exposure during sensitive developmental periods may affect infant development of motor, visual or auditory pathways. Alterations to the developmental timing or function of these pathways could potentially have profound effects on behavior and cognition in childhood.

Therefore, the goals of this work were three-fold. First, characterize the prenatal exposure of Chinese newborns to pesticides of all classes and identify predictors of those exposures. The second objective was to explore the effects of prenatal OP exposure on motor function in infancy, as measured by the Peabody developmental motor scales and infant neurological international battery. The final goal was to examine the extent to which prenatal OP exposure affects infant visual and auditory function, as measured by grating visual acuity and auditory brainstem response.

We found that season of birth was the strongest predictor of overall pesticide detects in cord blood. We also found deficits in global motor function, visual acuity, and head circumference in 9-month-old infants prenatally exposed to OPs. Chlorpyrifos was associated with statistically significant decrements in global motor function and visual acuity, as well as reduced head circumference. Naled was significantly inversely associated with fine motor function. Methamidophos was consistently associated with lower global motor function and slower auditory signal transmission across the entire study period. Phorate was not associated with the neurodevelopmental outcomes examined here, but was significantly associated with reduced head circumference. Of these commonly

used OPs, only chlorpyrifos had been studied for neurodevelopmental effects in humans prior to this study.

Early motor skill acquisition in infancy provides the foundation for non-verbal communication in infancy and cognitive and socio-emotional development in childhood. Similarly, visual and auditory system development in infancy is crucial for the development of language and other forms of communication, as well as reading skills in childhood. Therefore, disruption of motor or sensory systems maturation, possibly as a result of prenatal OP exposure, could potentially have detrimental long-term effects on learning or other cognitive functions in childhood. Even small, subclinical changes, that may seem negligible on an individual level, could have potentially detrimental effects at the population level.

CHAPTER I

INTRODUCTION

Neurodevelopmental disorders

One in six children living in industrialized countries suffers from a neurodevelopmental disorder, including difficulties with language, speech, motor skills, behavior, memory, learning, and other neurological functions (WHO, 2011). Some disorders, such as autism spectrum disorder (ASD) and attention-deficit/hyperactivity disorder (ADHD), appear to be increasing in prevalence over the last several decades (Grandjean & Landrigan, 2006; Landrigan, Lambertini, & Birnbaum, 2012; U.S. EPA, 2013; WHO, 2011). While long-term trends can be difficult to assess, due to a lack of historical data and changes in awareness and diagnostic criteria, the available evidence does support a long-term rise in autism (Grandjean & Landrigan, 2014; Grupp-Phelan, Harman, & Kelleher, 2007; Hertz-Picciotto & Delwiche, 2009; Newschaffer, 2006; U.S.CDC, 2009) and other behavioral and learning disorders (Grandjean & Landrigan, 2014; Grupp-Phelan, et al., 2007; Kelleher, McInerny, Gardner, Childs, & Wasserman, 2000; U.S. Dept. of Education, 2007).

Neurodevelopmental disorders can be detrimental to a child's quality of life, affecting behavior, and academic achievement, as well as having more long-term effects on workplace productivity and overall welfare (Bellinger, 2009).

Subclinical decrements in brain function, which may go undetected, may be even more common than these diagnosed disorders, however (Grandjean & Landrigan, 2014). While these small subclinical changes, such as a loss of a few IQ points, may be seem negligible on an individual level, they could have deleterious effects at the population level (Bellinger, 2009).

The triggers for the current "pandemic" of neurodevelopmental disorders are only partially understood. Most are thought to be highly multifactorial, with genetic and environmental risk factors contributing to their etiology. Genetic factors are believed to be responsible for around 30-40% of all neurodevelopmental disorders, however genetics alone cannot explain the increases in prevalence (Grandjean & Landrigan, 2014). Nearly ubiquitous exposure to chemicals in the environment is believed to play an important role in the global uptick of these disorders (Grandjean & Landrigan, 2014).

Synthetic pesticides

Synthetic pesticides have been implicated as possible contributors to the global rise in neurodevelopmental disorders. Synthetic pesticides are toxic to biological systems by design, with many of the most commonly employed classes utilizing a neurotoxic mode of action. For example, organophosphate (OP), pyrethroid (PYR), carbamate (CARB), and some organochlorine (OC) insecticides disrupt signaling in the central nervous system (CNS), thereby inhibiting neurological function (Abdollahi & Karami-Mohajeri, 2012; Yang &

Deng, 2007).

Synthetic pesticides are used extensively worldwide for pest management in a wide variety of agricultural, industrial, and residential settings. Globally 4.6 million tons of synthetic pesticides are applied each year within the agricultural sector (U.S. EPA, 2011; W. Zhang, Jiang, & JF., 2011). China is one of the world's largest consumers of pesticides (Ding & Bao, 2013; U.S. EPA, 2011; W. Zhang, et al., 2011), with over 300,000 tons applied to food crops each year (Y. Zhang, et al., 2014). The average amount of pesticides used in one field unit in China is up to five times higher than the global average (Y. Zhang, et al., 2014). Farmers overuse or improperly use pesticides in an attempt to improve crop yields, resulting in high residual levels at the time of harvest (Ding & Bao, 2013; Huang, Qiao, Zhang, & Rozelle, 2001). A survey of vegetable and fruit markets of China indicated that more than half of the vegetable samples had detectable pesticide residuals, many of which grossly exceeded the national standard (W. Zhang, et al., 2011). Additionally, 40% of pesticides on the market in China are sold under false brand names (Ding & Bao, 2013; PAN, 2003), likely also contributing to their misuse.

In addition to large-scale agricultural uses, pesticides are also employed for a variety of residential pest control applications, such as control of termites, ants or cockroaches (Horton, et al., 2011). They are used in topical treatments for lice, fleas and ticks. Pesticides have long been used for public health vector-control programs, controlling transmission of mosquito-borne infectious diseases (van den Berg, et al., 2011). Given this widespread use, exposure to synthetic

pesticides in the general population occurs via a multitude of pathways. Common routes of exposure include: consumption of food, water, soil or house dust contaminated with residual pesticides, inhalation of vapors or aerosols from spray drift, and dermal absorption if skin contact occurs (Costa, Giordano, Guizzetti, & Vitalone, 2008).

However, despite nearly ubiquitous pesticide exposures among the non-occupationally exposed population, there is a lack of data on the neurological health effects of low-level exposures. In particular, little is known about the potential short- and long-term neurodevelopmental effects of pesticide exposure *in utero* and in early childhood. Fetal and infant brains are rapidly developing, making them highly vulnerable to long-lasting effects of pesticide exposure (Garcia, Seidler, & Slotkin, 2005; Rice & Barone, 2000). Important windows of susceptibility occur *in utero*, during infancy, and in early childhood (V. A. Rauh & Margolis, 2016; Rice & Barone, 2000). Exposure during these highly vulnerable periods, even to low levels that would have no effect in adults, can lead to permanent brain injury or alterations in brain architecture or circuitry (Garcia, et al., 2005; Grandjean & Landrigan, 2014). Fetal susceptibility to pesticides is further increased by the fact that many pesticides can cross the placenta (Bradman, et al., 2003). Fetuses also have lower levels of detoxifying enzymes to metabolize pesticides (Eskenazi, et al., 2008) and immature metabolic pathways, which slow excretion (V. A. Rauh & Margolis, 2016). Yet despite these concerns, there are hundreds of pesticides in active use that have never been tested for neurodevelopmental toxicity (Grandjean & Landrigan, 2014).

Aim 1

China is the world's largest consumer of pesticides, but despite potential for a highly exposed population, little has been published about the levels of prenatal pesticide exposure in China. Previous studies of pesticides in cord blood have focused on the OC class of insecticides (Cao, et al., 2011; Li, et al., 2014; Zhao, Xu, Li, Han, & Ling, 2007), which have now largely been phased out from use. A few studies have concentrated on the more modern classes of non-persistent pesticides, either OPs (Liu, et al., 2016; Y. Zhang, et al., 2014) or PYR (Qi, et al., 2012; Xue, et al., 2013), but these are limited by the use of non-specific urinary metabolites for their exposure assessment, which make it difficult to attribute health effects to the parent pesticide. Analyses of predictors of prenatal pesticide exposure in China have also been limited to either OCs (Cao, et al., 2011; Lee, et al., 2007; Qi, et al., 2012; Xue, et al., 2013) or PYR (Qi, et al., 2012; Xue, et al., 2013).

Aim 1 of this dissertation sought to address some of these limitations in the literature by first characterizing the prenatal exposure of Chinese newborns to 96 pesticides of all classes by measuring the level of parent compound directly in cord blood. The second part of aim 1 was to identify which demographic characteristics predicted those prenatal pesticide exposures. I hypothesized that infants were exposed to wide range of pesticides and that parental occupation, family income, place of residence, and season of birth were predictors of that

exposure.

Organophosphate insecticides (OPs)

Aims 2 and 3 of this dissertation focus specifically on OPs, which account for more than a third of all insecticide use in China (Y. Zhang, et al., 2014). OPs are the most commonly used insecticide in China's agricultural sector (Ding & Bao, 2013). OPs are non-persistent in the environment and were therefore considered a safe alternative to the highly persistent OCs, such as dichloro-diphenyl-trichloroethane (DDT). However despite their quick degradation, OPs actually have much greater acute toxicity than the OCs they were replacing (Rana, 2006).

OPs have emerged as a particular concern for developmental neurotoxicity over the last couple decades. In 2001, the U.S. EPA banned the OPs, diazinon and chlorpyrifos, for residential use (U.S. EPA, 2011), and China similarly banned parathion, methamidophos, and phosphamidon in 2007 (W. Zhang, et al., 2011), because of concerns of neurotoxicity in infants. Despite this, OPs are still employed in farming in both countries, and are the most heavily used pesticides in the agricultural sector in China (Ding & Bao, 2013). Given their heavy use in agriculture, the main route of OP exposure in the general population is via the diet. More than 10% of fruits, vegetables, and cereal grains grown in China contain OP residues that exceed the national safety standards and banned OPs are regularly detected (Chen, et al., 2012; L. Wang, Liang, &

Jiang, 2008; S. Wang, Wang, Zhang, Wang, & Guo, 2013). Additional exposure to OPs may also occur via contaminated drinking water, dust, and spray drift (Huang, et al., 2001).

The mechanism of acute toxicity elicited by OPs is well understood. OPs inhibit acetylcholinesterase (AChE), the enzyme responsible for terminating the neurotransmitter acetylcholine's activity. Without the inhibition of AChE, acetylcholine builds up in the synapse, leading to hyperstimulation of the cholinergic receptors at neuronal and neuromuscular junctions (Abdollahi & Karami-Mohajeri, 2012; Eddleston, Buckley, Eyer, & Dawson, 2008; Kamanyire & Karalliedde, 2004). At low dose exposure levels, that do not elicit cholinergic toxicity or acetylcholinesterase inhibition, neurodevelopmental toxicity is still observed, however. In laboratory studies, chlorpyrifos, a well-known OP, has been found to disrupt neuronal processes such as neuron replication and differentiation, axon formation, synaptogenesis, apoptosis, and neural circuit formation, even at low doses where cholinergic toxicity is not present (Slotkin, 2004). Epidemiological studies also provide evidence of associations between prenatal exposure to OPs and neurological effects in childhood such as IQ deficits (Bouchard, et al., 2011; Engel, et al., 2011; V. Rauh, et al., 2011) and cognitive delays (Bouchard, et al., 2011; Engel, et al., 2011; Eskenazi, et al., 2007; V. Rauh, et al., 2011; V. A. Rauh, et al., 2006), as well as increased diagnoses of ASD (Shelton, et al., 2014), ADHD (Marks, et al., 2010; V. A. Rauh, et al., 2006), and pervasive developmental (Eskenazi, et al., 2007; V. A. Rauh, et al., 2006) disorders. Despite a growing body of evidence that prenatal OP

exposure can affect these oft-studied neurological and cognitive endpoints in childhood, much less is known about how it may affect infant development of motor, visual or auditory pathways. Any alteration to these developmental trajectories in infancy could potentially have profound effects on downstream behavior and cognition.

Aim 2

Motor skill acquisition in infancy provides the basis for cognitive and socio-emotional development in childhood (Clearfield, 2004, 2011), as well as supplying the foundation for non-verbal communication (Bhat, Galloway, & Landa, 2012). Motor functions improve rapidly in infancy with increasing CNS maturation and meeting early motor milestones serves as benchmark of healthy neurological development (De Felice, Scattoni, Ricceri, & Calamandrei, 2015; Noritz & Murphy, 2013). Epidemiological studies provide preliminary evidence that prenatal OP exposure may negatively affect infant or child motor-related outcomes. Prenatal OP exposure has been associated with deficits in reflexes (Engel, et al., 2007; Young, et al., 2005; Y. Zhang, et al., 2014), psychomotor development (V. A. Rauh, et al., 2006), fine motor skills (Handal, Harlow, Breilh, & Lozoff, 2008), and motor speed and coordination (Harari, et al., 2010). However many of these studies are limited in their exposure assessment, using either nonspecific maternal urinary metabolites during pregnancy (Engel, et al., 2007; Young, et al., 2005; Y. Zhang, et al., 2014) or self-reported maternal

occupational exposure during pregnancy (Handal, et al., 2008; Harari, et al., 2010).

Aim 2 of this dissertation sought to add to this literature by investigating the effects of prenatal exposure to five commonly used OPs, measured directly in cord blood, on infant motor function at two time points during infancy. Motor function was assessed using two highly specific tests, the Peabody Developmental Motor Scales, 2nd edition (PDMS-2), a global motor test, and the Infant Neurological International Battery (INFANIB), a neuromotor test. These tests are commonly used in clinical settings to identify issues with motor abilities in infancy and early childhood so that early interventions can be employed. I hypothesized that infants with prenatal exposure to OPs would have deficits in motor function, which would be evidenced by lower PDMS and INFANIB scores.

Aim 3

Proper visual and auditory system development in infancy is crucial to later learning processes such as the development of language and other forms of communication, as well providing the foundation for reading skills in childhood (Algarin, Peirano, Garrido, Pizarro, & Lozoff, 2003; Chonchaiya, et al., 2013). Only two epidemiological studies, to date, have examined the effects of prenatal OP exposure on visual or auditory-related outcomes (Handal, et al., 2008; Sturza, et al., 2016). Prenatal OP exposure was associated with significantly higher odds of poor visual acuity in infants (Handal, et al., 2008), while number of

pesticides (OPs and other classes) in cord blood was associated with slower auditory signal transmission in infants (Sturza, et al., 2016). These studies provide some preliminary evidence that prenatal OP exposure may negatively affect early-childhood sensory-related functions, but both are limited by their exposure assessments. One study used self-reported maternal occupational exposure during pregnancy to define exposure (Handal, et al., 2008), while the other used “number of detects” (of all classes) in cord blood to classify exposure (Sturza, et al., 2016), making it hard to attribute any effects to specific OPs. Additionally the Sturza, et al. study, a pilot study for the current work, had very limited sample size.

Therefore, Aim 3 of this dissertation sought to build on these studies by investigating the extent to which prenatal exposure to five OPs, measured directly in cord blood, affects visual and auditory function at three time points throughout infancy. Visual function was assessed using a test of grating visual acuity (VA), while auditory function was assessed using auditory brainstem response (ABR). These tests are used to clinically assess the intactness of visual and auditory pathways throughout infancy and early childhood. I hypothesized that infants with prenatal exposure to OPs would have deficits in visual and auditory function, which would be manifested by lower grating VA scores and longer ABR latencies, indicating slower auditory signal transmission.

Study population

This study was conducted in Zhejiang province, along the eastern coast of China. Agricultural pesticide use in Zhejiang is thought to be particularly high. Applications are reported to be nearly double the national rate (Huang, et al., 2001) which would translate to nearly 10 times the global average (Y. Zhang, et al., 2014). Study participants were all residents of Fuyang County, a largely rural area located southwest of the capital city, Hangzhou. Between 2008 and 2011, women with healthy, uncomplicated, singleton pregnancies were recruited at 37 to 42 weeks gestation and consented to cord blood screening. 359 healthy, full-term infants were enrolled at birth into a longitudinal study of iron deficiency and neurodevelopment. Pesticides were retroactively measured in stored cord blood samples.

References

Abdollahi, M., & Karami-Mohajeri, S. (2012). A comprehensive review on experimental and clinical findings in intermediate syndrome caused by organophosphate poisoning. *Toxicol Appl Pharmacol, 258*(3), 309-314.

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*(2), 217-223.

Bellinger, D. C. (2009). Interpreting epidemiologic studies of developmental neurotoxicity: conceptual and analytic issues. *Neurotoxicol Teratol, 31*(5), 267-274.

Bhat, A. N., Galloway, J. C., & Landa, R. J. (2012). Relation between early motor delay and later communication delay in infants at risk for autism. *Infant Behav Dev, 35*(4), 838-846.

Bouchard, M. F., Chevrier, J., Harley, K. G., Kogut, K., Vedar, M., Calderon, N., et al. (2011). Prenatal exposure to organophosphate pesticides and IQ in 7-year-old children. *Environ Health Perspect, 119*(8), 1189-1195.

Bradman, A., Barr, D. B., Claus Henn, B. G., Drumheller, T., Curry, C., & Eskenazi, B. (2003). Measurement of pesticides and other toxicants in amniotic fluid as a potential biomarker of prenatal exposure: a validation study. *Environ Health Perspect, 111*(14), 1779-1782.

Cao, L. L., Yan, C. H., Yu, X. D., Tian, Y., Zhao, L., Liu, J. X., et al. (2011). Relationship between serum concentrations of polychlorinated biphenyls and organochlorine pesticides and dietary habits of pregnant women in Shanghai. *Sci Total Environ, 409*(16), 2997-3002.

Chen, C., Qian, Y., Liu, X., Tao, C., Liang, Y., & Li, Y. (2012). Risk assessment of chlorpyrifos on rice and cabbage in China. *Regul Toxicol Pharmacol, 62*(1), 125-130.

Chonchaiya, W., Tardif, T., Mai, X., Xu, L., Li, M., Kaciroti, N., et al. (2013). Developmental trends in auditory processing can provide early predictions of language acquisition in young infants. *Dev Sci, 16*(2), 159-172.

Clearfield, M. W. (2004). The role of crawling and walking experience in infant spatial memory. *J Exp Child Psychol, 89*(3), 214-241.

Clearfield, M. W. (2011). Learning to walk changes infants' social interactions. *Infant Behav Dev, 34*(1), 15-25.

Costa, L. G., Giordano, G., Guizzetti, M., & Vitalone, A. (2008). Neurotoxicity of pesticides: a brief review. *Front Biosci, 13*, 1240-1249.

De Felice, A., Scattoni, M. L., Ricceri, L., & Calamandrei, G. (2015). Prenatal exposure to a common organophosphate insecticide delays motor development in a mouse model of idiopathic autism. *PLoS One, 10*(3), e0121663.

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

Eddleston, M., Buckley, N. A., Eyer, P., & Dawson, A. H. (2008). Management of acute organophosphorus pesticide poisoning. *Lancet, 371*(9612), 597-607.

Engel, S. M., Berkowitz, G. S., Barr, D. B., Teitelbaum, S. L., Siskind, J., Meisel, S. J., et al. (2007). Prenatal organophosphate metabolite and organochlorine levels and performance on the Brazelton Neonatal Behavioral Assessment Scale in a multiethnic pregnancy cohort. *Am J Epidemiol, 165*(12), 1397-1404.

Engel, S. M., Wetmur, J., Chen, J., Zhu, C., Barr, D. B., Canfield, R. L., et al. (2011). Prenatal exposure to organophosphates, paraoxonase 1, and cognitive development in childhood. *Environ Health Perspect, 119*(8), 1182-1188.

Eskenazi, B., Marks, A. R., Bradman, A., Harley, K., Barr, D. B., Johnson, C., et al. (2007). Organophosphate pesticide exposure and neurodevelopment in young Mexican-American children. *Environ Health Perspect, 115*(5), 792-798.

Eskenazi, B., Rosas, L. G., Marks, A. R., Bradman, A., Harley, K., Holland, N., et al. (2008). Pesticide toxicity and the developing brain. *Basic Clin Pharmacol Toxicol, 102*(2), 228-236.

Garcia, S. J., Seidler, F. J., & Slotkin, T. A. (2005). Developmental neurotoxicity of chlorpyrifos: targeting glial cells. *Environ Toxicol Pharmacol, 19*(3), 455-461.

Grandjean, P., & Landrigan, P. J. (2006). Developmental neurotoxicity of industrial chemicals. *Lancet, 368*(9553), 2167-2178.

Grandjean, P., & Landrigan, P. J. (2014). Neurobehavioural effects of developmental toxicity. *Lancet Neurol, 13*(3), 330-338.

Grupp-Phelan, J., Harman, J. S., & Kelleher, K. J. (2007). Trends in mental health and chronic condition visits by children presenting for care at U.S. emergency departments. *Public Health Rep, 122*(1), 55-61.

Handal, A. J., Harlow, S. D., Breilh, J., & Lozoff, B. (2008). Occupational exposure to pesticides during pregnancy and neurobehavioral development of infants and toddlers. *Epidemiology, 19*(6), 851-859.

Harari, R., Julvez, J., Murata, K., Barr, D., Bellinger, D. C., Debes, F., et al. (2010). Neurobehavioral deficits and increased blood pressure in school-age children prenatally exposed to pesticides. *Environ Health Perspect, 118*(6), 890-896.

Hertz-Picciotto, I., & Delwiche, L. (2009). The rise in autism and the role of age at diagnosis. *Epidemiology, 20*(1), 84-90.

Horton, M. K., Jacobson, J. B., McKelvey, W., Holmes, D., Fincher, B., Quantano, A., et al. (2011). Characterization of residential pest control products used in inner city communities in New York City. *J Expo Sci Environ Epidemiol, 21*(3), 291-301.

Huang, J., Qiao, F., Zhang, L., & Rozelle, S. (2001). *Farm pesticide, rice production, and human health*.

Kamanyire, R., & Karalliedde, L. (2004). Organophosphate toxicity and occupational exposure. *Occup Med (Lond), 54*(2), 69-75.

Kelleher, K. J., McInerny, T. K., Gardner, W. P., Childs, G. E., & Wasserman, R. C. (2000). Increasing identification of psychosocial problems: 1979-1996. *Pediatrics, 105*(6), 1313-1321.

Landrigan, P. J., Lambertini, L., & Birnbaum, L. S. (2012). A research strategy to discover the environmental causes of autism and neurodevelopmental disabilities. *Environ Health Perspect, 120*(7), a258-260.

Lee, S. A., Dai, Q., Zheng, W., Gao, Y. T., Blair, A., Tessari, J. D., et al. (2007). Association of serum concentration of organochlorine pesticides with dietary intake and other lifestyle factors among urban Chinese women. *Environ Int, 33*(2), 157-163.

Li, C., Cheng, Y., Tang, Q., Lin, S., Li, Y., Hu, X., et al. (2014). The association between prenatal exposure to organochlorine pesticides and thyroid hormone levels in newborns in Yancheng, China. *Environ Res, 129*, 47-51.

Liu, P., Wu, C., Chang, X., Qi, X., Zheng, M., & Zhou, Z. (2016). Adverse Associations of both Prenatal and Postnatal Exposure to Organophosphorous Pesticides with Infant Neurodevelopment in an Agricultural Area of Jiangsu Province, China. *Environ Health Perspect, 124*(10), 1637-1643.

Marks, A. R., Harley, K., Bradman, A., Kogut, K., Barr, D. B., Johnson, C., et al. (2010). Organophosphate pesticide exposure and attention in young Mexican-American children: the CHAMACOS study. *Environ Health Perspect, 118*(12), 1768-1774.

Newschaffer, C. J. (2006). Investigating diagnostic substitution and autism prevalence trends. *Pediatrics, 117*(4), 1436-1437.

Noritz, G. H., & Murphy, N. A. (2013). Motor delays: early identification and evaluation. *Pediatrics, 131*(6), e2016-2027.

PAN. (2003). Pesticide Residues a Major Threat to China's Ag Exports. 2014, from http://www.panna.org/legacy/panups/panup_20030117.dv.html

Qi, X., Zheng, M., Wu, C., Wang, G., Feng, C., & Zhou, Z. (2012). Urinary pyrethroid metabolites among pregnant women in an agricultural area of the Province of Jiangsu, China. *Int J Hyg Environ Health, 215*(5), 487-495.

Rana, S. (2006). *Environmental Pollution: Health and Toxicology*. Oxford, U.K.: Alpha Science International Ltd.

Rauh, V., Arunajadai, S., Horton, M., Perera, F., Hoepner, L., Barr, D. B., et al. (2011). Seven-year neurodevelopmental scores and prenatal exposure to chlorpyrifos, a common agricultural pesticide. *Environ Health Perspect, 119*(8), 1196-1201.

Rauh, V. A., Garfinkel, R., Perera, F. P., Andrews, H. F., Hoepner, L., Barr, D. B., et al. (2006). Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. *Pediatrics, 118*(6), e1845-1859.

Rauh, V. A., & Margolis, A. E. (2016). Research Review: Environmental exposures, neurodevelopment, and child mental health - new paradigms for the study of brain and behavioral effects. *J Child Psychol Psychiatry, 57*(7), 775-793.

Rice, D., & Barone, S., Jr. (2000). Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. *Environ Health Perspect, 108 Suppl 3*, 511-533.
Shelton, J. F., Geraghty, E. M., Tancredi, D. J., Delwiche, L. D., Schmidt, R. J., Ritz, B., et al. (2014). Neurodevelopmental disorders and prenatal residential proximity to agricultural pesticides: the CHARGE study. *Environ Health Perspect, 122*(10), 1103-1109.
Slotkin, T. A. (2004). Guidelines for developmental neurotoxicity and their impact on organophosphate pesticides: a personal view from an academic perspective. *Neurotoxicology, 25*(4), 631-640.
Sturza, J., Silver, M. K., Xu, L., Li, M., Mai, X., Xia, Y., et al. (2016). Prenatal exposure to multiple pesticides is associated with auditory brainstem response at 9months in a cohort study of Chinese infants. *Environ Int, 92-93*, 478-485.
U.S. CDC. (2009). Prevalence of autism spectrum disorders - autism and developmental disabilities monitoring network, United States, 2006. *Morbidity and Mortality Weekly Report 58*(SS 10), 1-20.
U.S. Dept. of Education. (2007). *27th Annual (2005) Report to Congress on the Implementation of the Individuals with Disabilities Education Act*.
U.S. EPA. (2011). Pesticide industry sales and usage: 2006 and 2007 market estimates. Retrieved March 13, 2013, from http://www.panna.org/sites/default/files/EPA%20market_estimates2007.pdf
U.S. EPA. (2013). *America's Children and the Environment: Neurodevelopmental Disorders*.
van den Berg, H., Hii, J., Soares, A., Mnzava, A., Ameneshewa, B., Dash, A. P., et al. (2011). Status of pesticide management in the practice of vector control: a global survey in countries at risk of malaria or other major vector-borne diseases. *Malar J, 10*, 125.
Wang, L., Liang, Y., & Jiang, X. (2008). Analysis of eight organophosphorus pesticide residues in fresh vegetables retailed in agricultural product markets of Nanjing, China. *Bull Environ Contam Toxicol, 81*(4), 377-382.
Wang, S., Wang, Z., Zhang, Y., Wang, J., & Guo, R. (2013). Pesticide residues in market foods in Shaanxi Province of China in 2010. *Food Chem, 138*(2-3), 2016-2025.
WHO. (2011). *Children and neurodevelopmental behavioral intellectual disorders*.
Xue, Z., Li, X., Su, Q., Xu, L., Zhang, P., Kong, Z., et al. (2013). Effect of synthetic pyrethroid pesticide exposure during pregnancy on the growth and development of infants. *Asia Pac J Public Health, 25*(4 Suppl), 72S-79S.
Yang, C. C., & Deng, J. F. (2007). Intermediate syndrome following organophosphate insecticide poisoning. *J Chin Med Assoc, 70*(11), 467-472.
Young, J. G., Eskenazi, B., Gladstone, E. A., Bradman, A., Pedersen, L., Johnson, C., et al. (2005). Association between in utero organophosphate

pesticide exposure and abnormal reflexes in neonates. *Neurotoxicology, 26*(2), 199-209.
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*(2), 125-144.
Zhang, Y., Han, S., Liang, D., Shi, X., Wang, F., Liu, W., et al. (2014). Prenatal exposure to organophosphate pesticides and neurobehavioral development of neonates: a birth cohort study in shenyang, china. *PLoS One, 9*(2), e88491.
Zhao, G., Xu, Y., Li, W., Han, G., & Ling, B. (2007). Prenatal exposures to persistent organic pollutants as measured in cord blood and meconium from three localities of Zhejiang, China. *Sci Total Environ, 377*(2-3), 179-191.

CHAPTER II

AIM 1: Distribution and predictors of pesticides in the umbilical cord blood of Chinese newborns

Abstract

Introduction: Rates of pesticide use in Chinese agriculture are five times greater than the global average, leading to high exposures via the diet. Many are neurotoxic, making prenatal pesticide exposure a concern. Previous studies of prenatal exposure in China focused almost entirely on organochlorines. Here study goals were to characterize exposure of Chinese newborns to all classes of pesticides and identify predictors of those exposures.

Methods: 84 pesticides and 12 metabolites were measured in umbilical cord plasma of 336 infants. Composite variables were created for total detects overall and by class. Individual pesticides were analyzed as dichotomous or continuous, based on detection rates. Relationships between demographic characteristics and pesticides were evaluated using generalized linear regression.

Results: 75 pesticides were detected. The mean (SD) detected per sample was 15.3 (6.1). Increased pesticide detects were found in cord blood of infants born in the summer (β=2.2, p=0.01), particularly July (β=4.0, p=0.03). Similar trends were observed for individual insecticide classes.

Conclusions: A summer birth was the strongest predictor of pesticide detects in cord blood. Associations were more striking for overall pesticide exposure than for individual pesticides, highlighting the importance of considering exposures to mixtures of pesticides, rather than individual agents or classes.

Introduction

Globally, nearly five million tons of synthetic pesticides are applied agriculturally each year (U.S.EPA, 2011; W. Zhang, Jiang, & JF., 2011). China, one of the world's largest consumers of pesticides (Ding & Bao, 2013; U.S. EPA, 2011; W. Zhang, et al., 2011), applies over 300,000 tons to food crops annually, more than 2.5- to 5-fold higher than the global average per field unit (Y. Zhang, et al., 2014). Rates in Zhejiang province, where this study was conducted, are some of the highest in China, at nearly double the national rate (Huang, Qiao, Zhang, & Rozelle, 2001). Farmers are thought to overuse or improperly use pesticides in an attempt to improve crop yields, resulting in high residual levels at the time of harvest (Ding & Bao, 2013; Huang, et al., 2001).

Due to prolific pesticide use in agriculture, the most common route of non-occupational exposure to pesticides is via consumption of contaminated food (Huang, et al., 2001). Additional related exposures may also occur via contaminated drinking water and spray drift, especially in rural, farming communities, or from the use of residential pesticides in the home or yard (Huang, et al., 2001). Organophosphate (OP) insecticides are the most heavily

used agricultural pesticide in China, while pyrethroid (PYR) insecticides are the most commonly used pesticides in residential settings (Ding & Bao, 2013).

Many pesticides, and particularly insecticides, act by disrupting signaling mechanisms in the central nervous system (CNS), thereby inhibiting neurological function. Because of their neurotoxic mode of action, pesticides have been implicated as possible contributors to the rise in neurodevelopmental disorders among children (Rosas & Eskenazi, 2008). Infant and fetal brains are rapidly developing, making them vulnerable to long-lasting effects of pesticide exposure, such as disruption of brain architecture or circuitry (Garcia, Seidler, & Slotkin, 2005). 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 characteristics are thought to increase fetal susceptibility.

Despite having the world's largest population coupled with the potential for high exposures, relatively little has been published about the levels of prenatal pesticide exposure in China. Five studies reported pesticide levels in umbilical cord blood (Cao, et al., 2011; Guo, et al., 2014; Li, et al., 2014; Wickerham, et al., 2012; Zhao, Xu, Li, Han, & Ling, 2007), while others examined maternal urinary metabolites during pregnancy (Qi, et al., 2012; Xue, et al., 2013; Y. Zhang, et al., 2014). Of the five cord blood studies, only one measured pesticides of varying classes (insecticides, herbicides, fungicides, and repellant) (Wickerham, et al., 2012); all others focused solely on the organochlorine (OC) class of insecticides.

Our exposure assessment extends these studies by examining 96 pesticides and metabolites from a wide variety of classes, enabling us to begin to

consider the real world problem of multiple, concurrent pesticide exposures. The goals of this study were to characterize pesticide exposures among Chinese newborns and identify predictors of those exposures. This work lays the foundation for future work examining prenatal pesticide exposure and infant neurodevelopment in our cohort.

Methods

Ethics statement

Institutional review board approval was obtained from the ethics committees of the University of Michigan and Children's Hospital Zhejiang University. Signed, informed consent was obtained from parents.

Study population

Pesticide analysis was performed for 336 infants from rural Fuyang county near Hangzhou, China in Zhejiang province. Pregnant women with healthy, uncomplicated, singleton pregnancies were recruited between 2008 and 2011 from Fuyang Maternal and Children's hospital at 37-42 weeks gestation and consented to cord blood screening (n=1187). Of these infants, a subset (n=359) was then enrolled into a study of iron deficiency and infant neurodevelopment. The subset for neurodevelopmental assessment was selected based on cord blood iron status (low, marginal, normal) and parental consent for the developmental study. Of those, 237 had a sufficient volume of cord blood available for pesticide analysis. The remaining pesticide analysis samples

(n=99) were randomly selected from those with sufficient cord blood volume from the original cord blood screening cohort.

Pesticides

Following delivery, 20-30mL of cord blood was collected and immediately frozen. Blood samples were transferred twice weekly on dry ice from Fuyang to Hangzhou, where they were separated and stored at -80C at Children's Hospital Zhejiang University. Funding was obtained for the pesticide study in 2012. Plasma samples were transferred on dry ice to the Institute of Toxicology at Nanjing Medical University for pesticide analysis. Pesticides were chosen based on usage data, concerns of neurotoxicity, method compatibility, and pilot data. We analyzed 96 compounds (84 pesticides and 12 metabolites): 24 organophosphate (OP) insecticides, 6 OP metabolites, 12 pyrethroid (PYR) insecticides, 1 PYR metabolite, 3 carbamate (CARB) insecticides, 5 organochlorine (OC) insecticides, 3 OC metabolites, 5 miscellaneous insecticides of undetermined classes, 14 fungicides, 2 fungicide metabolites, 18 herbicides, and 3 "other-use" chemicals/synergists.

The pesticide analysis protocol was modified from previously published methods (Perez, et al., 2010; ThermoScientific). 800 µl plasma samples were mixed with 800 µL saturated ammonium sulfate. After centrifugation, the supernatant was subjected to solid-phase extraction (SPE) for cleaning and pre-concentration. The sample was loaded onto a conditioned and equilibrated ProElut C18 SPE cartridge (200 mg/3 mL; 50/pk, Dikma, China). After cleaning, analytes were harvested by eluting with dichloromethane and n-hexane. The

SPE eluate was concentrated and reconstituted into 10 µL toluene prior to GC-MS/MS analysis. The pesticides in serum were then separated with a Thermo Scientific TRACE GC Ultra gas chromatograph equipped with a column of TR-PESTICIDE II (30m, 0.25mm, 0.25µm) and measured in timed-SRM mode with a triple quadrupole TSQ XLS mass spectrometer (QqQ, Thermo Fisher Scientific, Inc., USA). Limits of detection (LODs) were determined by analyzing fortified serum on a signal-to-noise (S/N) ratio of three. Quality control samples were generated using blood samples with 0.675 and 1.35 ng/mL pesticide standards. Quality control samples and blanks were analyzed in parallel with study samples in each batch.

Given the likelihood of multiple concurrent pesticide exposures, we created several composite exposure variables. As a preliminary step, we dichotomized exposure to each pesticide. Concentrations below the limit of detection (< LOD) were assigned a value of 0, and those ≥LOD were assigned a value of 1. To assess overall pesticide exposure, we summed these dichotomous variables to create two indices of exposure for each infant: total number of detects and total number of detects not including metabolites. Because certain classes of pesticides may have similar modes of action and shared target sites within the body, we also created composites by class, summing the total number of exposures for each of the following: insecticides, non-persistent insecticides (no OCs), OPs, PYRs, fungicides, and herbicides (Figure II.1).

Individual pesticides were also analyzed as continuous variables when detection rates were ≥80% (values <LOD were replaced with LOD/√2) or dichotomous variables (detect/non-detect), when detection rates were 10-79%.

Predictors

Demographic and other variables analyzed as possible predictors of pesticide exposure were determined by maternal interview at the infant's 6-week follow-up visit. Household variables included: number of family members living in home, total number of people living in home, amount of living space in square meters, place of residence (rural/urban), and annual income (<30,000/30,000-49,999/50,000-99,999/≥100,000 Yuan). Parental characteristics included maternal and paternal age in years, education (middle school or less/high school or secondary school/college), and occupation (maternal: housewife/other; paternal: professional or administrator/manager/factory worker/other). Date of birth was used to create a season of birth variable (March-May/June-September/October-December) as well as a month of birth variable. All of the variables described here were analyzed as possible predictors of pesticide exposure.

Statistical analysis

Statistical analyses were conducted using SAS 9.3 (Cary, North Carolina). Descriptive statistics and frequencies for all variables of interest were examined. Percentile tables were created to determine the individual pesticide distributions within the sample. Generalized linear models (GLM) were used to assess relations between predictors and composite pesticide variables, as well as

individual pesticides or metabolites with detection rates ≥80%. Logistic regression models were used to assess relations between predictors and individual pesticides or metabolites with detection rates 10-79% (detect/non-detect).

Results

75 of the 96 pesticides and metabolites analyzed were detectable in at least one cord blood sample. The number of pesticides detected for individuals in the study population ranged from 0 to 48 with a mean (SD) of 15.3 (6.1) (Figure II.2). Excluding metabolites, the number of overall detects ranged from 0 to 41 with a mean (SD) of 12.5 (4.7). For total insecticides the range was 0 to 26 with a mean (SD) of 10.7 (3.7) (Figure II.2). The distributions of all detectable pesticides and their LODs are shown in Table 1. LODs ranged from 0.003 to 6.08 ng/mL, but the majority were well below 1 ng/mL. Quality control analysis yielded coefficients of variation ranging from 5-34%.

PYR and OP insecticides were the most commonly detected pesticides, with mean (SD) detects of 4.5 (2.1) and 3.4 (1.7), respectively. Complete distributions of pesticide concentrations are shown in Table II.1. Propoxur, a CARB insecticide, was the most commonly detected pesticide, found in all but two of the cord blood samples. Three pesticides and one metabolite were detected in ≥80% of the samples (naled, propoxur, aldrin, 3-phenoxybenzoic acid). Undetected pesticides (by class) included: OP- dicrotophos, dimethoate, formothion, phosphamidon, dimethylvinphos, methyl parathion, malathion,

dichlorvos; CARB- bendiocarb; fungicide- pyrimethanil, vinclozolin; fungicide metabolite- pentachloroaniline; herbicide- dimethipin, monolinuron, clomazone, isocarbamide, propyzamide, terbacil, dimethenamid, metribuzin, alachlor.

Characteristics of the study population are presented in Table II.2. Two-thirds of the study population lived in a rural area. Around 40% of mothers and fathers had a middle school education or less. The most common maternal and paternal occupations were housewife and professional/administrative, respectively. Despite the majority living in a rural area, only 4% of families had at least one parent classified as a rural worker, which included farmers, forestry workers, fishermen, and animal caretakers. No infants were born during January or February since study enrollment did not occur around the Chinese New Year holiday.

Infants born during the summer months of June to September had an average of 2.2 more pesticides detected in their cord blood than infants born between October and December (p=0.01) (Table II.3). By month of birth, July was the strongest predictor of overall pesticide exposure (4.0 more pesticides detected, on average, than for December, p=0.03). No other household, demographic, or parental characteristics appeared to influence overall pesticide exposure in our population (Appendix: Table II.A1).

Analyses of individual classes of pesticides similarly revealed that infants born in the summer had higher number of pesticides detected in their cord blood (Table II.3). Infants born between June and September had an average of 1.2 more insecticides, 0.4 more OPs, 0.7 more PYRs, and 0.2 more herbicides

detected in their cord blood than babies born between October and December (p=0.03, 0.09, 0.03, and 0.05, respectively). July was again the strongest predictor of insecticide exposure. Infants born in July had an average of 2.1 more insecticides, 1.5 more PYRs, and 0.7 more herbicides detected in their cord blood than infants born in December (p=0.04, 0.02, and 0.004, respectively). There were higher numbers of fungicides found in the cord blood of infants born in the spring, and the months of April, June, and October.

Significant predictors of highly detected (>50% detection) individual pesticides are shown in Table II.4. Key findings are summarized here. On average, 3-phenoxybenzoic acid concentrations increased by 1.3 ng/mL for every year increase in maternal age (p= 0.05). Women who were not housewives had lower odds of detectable methamidophos, compared to housewives. Odds of detecting prothiophos were significantly higher in the spring, while odds of detecting *trans*-permethrin, and trichlorfon were significantly higher in the summer, when compared to fall/winter. Naled concentrations were also significantly higher in the summer, while propoxur and 3-phenoxybenzoic acid concentrations were significantly lower in the spring, compared to the fall/winter. Additional significant findings for pesticides with lower detection rates (10-50%) can be found in the Appendix (Table II.A2).

Discussion

We found evidence of prenatal exposure to 75 pesticides or pesticide metabolites in a cohort of Chinese newborns in Zhejiang Province. Neonates, on

average, had detectable levels of 15 pesticides in their cord blood. Season of birth, specifically summer, was the strongest predictor of increased number of detects in cord blood. Infants born in July had significantly greater detects of cord pesticides than infants born in December. Similar trends were observed for individual classes of insecticides.

Until now, no study of this scope has been completed in China or elsewhere. A few relevant studies in the U.S. have measured 14-29 pesticides of all classes in cord blood (Neta, et al., 2010; Whyatt, et al., 2003; Yan, et al., 2009). Previous Chinese studies using cord blood analyzed a limited number of pesticides within a single class, reporting levels of 6-18 OC insecticides (Cao, et al., 2011; Guo, et al., 2014; Li, et al., 2014; Zhao, et al., 2007). Only our own pilot study reported cord blood levels for mixed classes (OPs, CARBs, herbicides, fungicides) (Wickerham, et al., 2012). Chinese studies of other common classes of insecticides, such as OPs and PYRs, have used maternal non-specific urinary metabolites during pregnancy as biomarkers of prenatal exposure (Qi, et al., 2012; Xue, et al., 2013; Y. Zhang, et al., 2014).

Of the pesticides measured in the present study, 29 were measured in previous U.S. (Neta, et al., 2010; Whyatt, et al., 2003; Yan, et al., 2009) or Chinese studies (Cao, et al., 2011; Guo, et al., 2014; Li, et al., 2014; Wickerham, et al., 2012; Zhao, et al., 2007). Table II.5 compares the high ends of the exposure distributions across the studies. In general, the 90th percentile concentrations in the Chinese studies are several orders of magnitude higher than the maximums reported in the U.S. studies. For example, for *cis*-

permethrin, a common pyrethroid insecticide, the 90th percentile for the current study was 28.32 ng/mL, while the comparable U.S. values were 0.001-0.010 ng/mL (Neta, et al., 2010; Whyatt, et al., 2003; Yan, et al., 2009). The pattern was similar for all pyrethroids, as well as many other pesticides. Thus, it appears that some infants in our study population were prenatally exposed to very high levels or pesticides compared to U.S. infants. However, we did not detect exposure to certain pesticides reported in U.S. or other Chinese studies. These included dichlorvos, malathion, methyl parathion, bendiocarb, vinclozilin, and alachlor. Dichlorvos and bendiocarb have never been measured in China before and may not be used there, or perhaps our methods were not sufficiently sensitive to detect them. Malathion, methyl parathion, vinclozilin, and alachlor were previously detected in our pilot work (Wickerham, et al., 2012). It is unclear why they were not detected with updated analytical methods.

Several prior studies also analyzed demographic characteristics as predictors of OC or PYR exposure in China. An exposure assessment of OCs in women of childbearing age reported lower OC levels in women with higher income and education (Lee, et al., 2007), while a study of OCs in cord blood found the opposite (Cao, et al., 2011). Two studies of PYR exposure in pregnant women found that maternal education was inversely related to PYR, with PYR urinary metabolites decreasing with higher education level (Qi, et al., 2012; Xue, et al., 2013). Positive associations between PYR metabolites and work as a manual laborer were reported for both studies (Qi, et al., 2012; Xue, et al., 2013) and with being housewife in one study (Qi, et al., 2012). In contrast, we did not

observe any significant associations between overall cord pesticide levels, OC, or PYR exposure and either income or maternal education. The number of fungicides detected were slightly lower in infants whose fathers had a secondary school versus college education, but this may be a chance finding. There were no noticeable trends of pesticide exposure by category of parental occupation, though non-housewives were slightly less exposed overall to OPs and had lower odds of detection for methamidophos. However, we had to rely on relatively broad, non-specific occupation categories and a dichotomous exposure metric (detect/non-detect). Finally, we did not find higher exposures in rural versus urban areas in contrast to a previous study of PYR exposure (Xue, et al., 2013). No previous studies analyzed predictors of OP, CARB, herbicide, or fungicide prenatal exposure in China.

Season of birth is a relatively unexplored predictor of prenatal pesticide exposure in China. One previous study reported higher levels of PYR urinary metabolites in pregnant women in June through September (Qi, et al., 2012), and pesticide poisonings are most commonly reported in August and September in Zhejiang province, which coincides with the farming season (M. Zhang, et al., 2013). In our study, season of birth was the strongest and most consistent predictor of cord pesticides. Total number of detects, total insecticides, and total OP, PYR, and fungicide detects were all higher in the cord blood of infants born in the summer months of June to September, compared to those born between October and December. Findings for individual pesticides also varied significantly by season and specific month. Although we were unable to find any

data on seasonal or monthly pesticide usage in China, it seems likely that these levels correlate with typical time of pesticide applications both agriculturally and residentially.

There were some additional limitations to this work. Because we measured a large number of pesticides with widely varying properties, the methods were not fully optimized for certain pesticides or classes of pesticides. This likely resulted in higher detection limits for some pesticides, compared to a more targeted approach. We were also unable to quantify exposure to some common pesticides and metabolites of interest, due to limitations in optimizing this high throughput methodology to all chemicals of interest. Generally speaking, analysis of pesticides in blood can result in a high frequency of non-detects, since pesticide levels in blood tend to be low, particularly for non-persistent pesticides that are rapidly metabolized (Barr, et al., 1999). While our optimized GC-MS/MS methods helped to minimize this concern, we still had large numbers of non-detects, limiting our ability to analyze pesticides on an individual basis, and necessitating the use of crude measures of exposure, such as number of detects by pesticide class. This approach is limited because it may not reflect dose or level of exposure. Additionally, funding for pesticide analyses was not received until a year after cord blood collection was completed. Pesticides are biologically reactive and may break down over time (Barr, et al., 1999; Munoz-Quezada, et al., 2013), although our blood collection and storage protocols were carefully designed to minimize these effects. Similarly, most of the pesticides measured here, with the exception of OC insecticides, were non-

persistent. With only one measure of exposure, we were unable to address the temporal variability of exposure during pregnancy. Another limitation is that we did not have data on lipid levels to adjust OC insecticide concentrations, as is the standard. Furthermore, because this was not originally designed as an environmental exposure study, we did not have information about residential pesticide use, maternal diet during pregnancy, and proximity to agriculture, which would have made this study more robust. Infants were not enrolled during the Chinese New Year holiday season (January-February), which limits our data on prenatal pesticide exposures during those winter months. Finally, the pesticide levels reported here for our infants from Zhejiang Province may not be representative of newborns elsewhere in China.

Despite its limitations, this study has important strengths. To our knowledge, it is the largest and most comprehensive exposure assessment of prenatal pesticide exposure anywhere in the world to date. The use of umbilical cord blood, as opposed to non-specific urinary metabolites, provides unequivocal evidence of fetal exposure (Barr, et al., 1999; Munoz-Quezada, et al., 2013) and may be more likely to reflect the available dose, since the measured dose was not yet eliminated from the infant's body (Needham, Ashley, & Patterson, 1995). These considerations are relevant for assessing and managing risk. Our analysis of predictors of prenatal exposure is more comprehensive than in previous Chinese studies. Our findings that associations between season of birth and exposure were more striking for overall pesticide exposure than for individual pesticides provide an important first step in highlighting the importance

of considering exposures to mixtures of chemicals, rather than focusing solely on individual agents or classes.

Conclusions

In conclusion, we reported pesticide exposure profiles in cord blood for 336 Chinese infants. 75 of 96 possible pesticides/metabolites were detected in at least one sample. On average, the infants had 15 pesticides detected in their cord blood samples, with some having as many as 48. Infants born in the summer months, especially in July, had greater numbers of detected pesticides in their cord blood, compared to infants born in the winter. Levels for many of the pesticides measured here, and particularly the pyrethroid insecticides, were orders of magnitude higher than those reported in cord blood in U.S. studies. Prenatal pesticide exposure is a concern, because the fetal brain is rapidly developing *in utero* and pesticide exposure during this period of critical development may have long-lasting effects on neurodevelopment. Many of the pesticides included in this analysis are proven or suspected developmental toxicants and future work in this cohort will seek to further elucidate the relationships between prenatal pesticide exposure and infant neurodvelopment. Although Chinese infants may be some of the most highly exposed in the world, due to high rates of pesticide use in Chinese agriculture, the pesticides targeted in this study are used worldwide.

Acknowledgments

This study was funded by R01ES021465 from the National Institute of Environmental Health Sciences (NIEHS), P01HD39386 from the National Institute of Child Health and Development (NICHD), and 81273085 from the National Natural Science Foundation of China (NNSFC).

I would also like to acknowledge my collaborators and coauthors on this work: Jie Shao, Minjian Chen, Yankai Xia, Betsy Lozoff and John Meeker.

References

Barr, D. B., Barr, J. R., Driskell, W. J., Hill, R. H., Jr., Ashley, D. L., Needham, L. L., et al. (1999). Strategies for biological monitoring of exposure for contemporary-use pesticides. *Toxicol Ind Health, 15*(1-2), 168-179.

Bradman, A., Barr, D. B., Claus Henn, B. G., Drumheller, T., Curry, C., & Eskenazi, B. (2003). Measurement of pesticides and other toxicants in amniotic fluid as a potential biomarker of prenatal exposure: a validation study. *Environ Health Perspect, 111*(14), 1779-1782.

Cao, L. L., Yan, C. H., Yu, X. D., Tian, Y., Zhao, L., Liu, J. X., et al. (2011). Relationship between serum concentrations of polychlorinated biphenyls and organochlorine pesticides and dietary habits of pregnant women in Shanghai. *Sci Total Environ, 409*(16), 2997-3002.

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

Eskenazi, B., Rosas, L. G., Marks, A. R., Bradman, A., Harley, K., Holland, N., et al. (2008). Pesticide toxicity and the developing brain. *Basic Clin Pharmacol Toxicol, 102*(2), 228-236.

Garcia, S. J., Seidler, F. J., & Slotkin, T. A. (2005). Developmental neurotoxicity of chlorpyrifos: targeting glial cells. *Environ Toxicol Pharmacol, 19*(3), 455-461.

Guo, H., Jin, Y., Cheng, Y., Leaderer, B., Lin, S., Holford, T. R., et al. (2014). Prenatal exposure to organochlorine pesticides and infant birth weight in China. *Chemosphere, 110*, 1-7.

Hou, R. L., Jin, W. Y., Chen, X. Y., Jin, Y., Wang, X. M., Shao, J., et al. (2014). Cord blood C-peptide, insulin, HbA1c, and lipids levels in small- and large-for-gestational-age newborns. *Med Sci Monit, 20*, 2097-2105.

Huang, J., Qiao, F., Zhang, L., & Rozelle, S. (2001). *Farm pesticide, rice production, and human health*.

Lee, S. A., Dai, Q., Zheng, W., Gao, Y. T., Blair, A., Tessari, J. D., et al. (2007). Association of serum concentration of organochlorine pesticides with dietary intake and other lifestyle factors among urban Chinese women. *Environ Int, 33*(2), 157-163.

Li, C., Cheng, Y., Tang, Q., Lin, S., Li, Y., Hu, X., et al. (2014). The association between prenatal exposure to organochlorine pesticides and thyroid hormone levels in newborns in Yancheng, China. *Environ Res, 129*, 47-51.

Munoz-Quezada, M. T., Lucero, B. A., Barr, D. B., Steenland, K., Levy, K., Ryan, P. B., et al. (2013). Neurodevelopmental effects in children associated with exposure to organophosphate pesticides: a systematic review. *Neurotoxicology, 39*, 158-168.

Needham, L. L., Ashley, D. L., & Patterson, D. G., Jr. (1995). Case studies of the use of biomarkers to assess exposures. *Toxicol Lett, 82-83*, 373-378.

Neta, G., Goldman, L. R., Barr, D., Sjodin, A., Apelberg, B. J., Witter, F. R., et al. (2010). Distribution and determinants of pesticide mixtures in cord serum using principal component analysis. *Environ Sci Technol, 44*(14), 5641-5648.

Perez, J. J., Williams, M. K., Weerasekera, G., Smith, K., Whyatt, R. M., Needham, L. L., et al. (2010). Measurement of pyrethroid, organophosphorus, and carbamate insecticides in human plasma using isotope dilution gas chromatography-high resolution mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci, 878*(27), 2554-2562.

Qi, X., Zheng, M., Wu, C., Wang, G., Feng, C., & Zhou, Z. (2012). Urinary pyrethroid metabolites among pregnant women in an agricultural area of the Province of Jiangsu, China. *Int J Hyg Environ Health, 215*(5), 487-495.

Rosas, L. G., & Eskenazi, B. (2008). Pesticides and child neurodevelopment. *Curr Opin Pediatr, 20*(2), 191-197.

Rylander, L., Nilsson-Ehle, P., & Hagmar, L. (2006). A simplified precise method for adjusting serum levels of persistent organohalogen pollutants to total serum lipids. *Chemosphere, 62*(3), 333-336.

ThermoScientific. Rapid analysis of pesticides in difficult matrices using GC/MS/MS. Retrieved September 15, 2013, from http://www.analiticaweb.com.br/newsletter/09/51880_RapidAnalysisPesticides.pdf

U.S. EPA. (2011). Pesticide industry sales and usage: 2006 and 2007 market estimates. Retrieved March 13, 2013, from http://www.panna.org/sites/default/files/EPA%20market_estimates2007.pdf

Whyatt, R. M., Barr, D. B., Camann, D. E., Kinney, P. L., Barr, J. R., Andrews, H. F., et al. (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*(5), 749-756.

Wickerham, E. L., Lozoff, B., Shao, J., Kaciroti, N., Xia, Y., & Meeker, J. D. (2012). Reduced birth weight in relation to pesticide mixtures detected in cord blood of full-term infants. *Environ Int, 47*, 80-85.

Xue, Z., Li, X., Su, Q., Xu, L., Zhang, P., Kong, Z., et al. (2013). Effect of synthetic pyrethroid pesticide exposure during pregnancy on the growth and development of infants. *Asia Pac J Public Health, 25*(4 Suppl), 72S-79S.

Yan, X., Lashley, S., Smulian, J. C., Ananth, C. V., Barr, D. B., Ledoux, T. A., et al. (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*(5), 948-967.

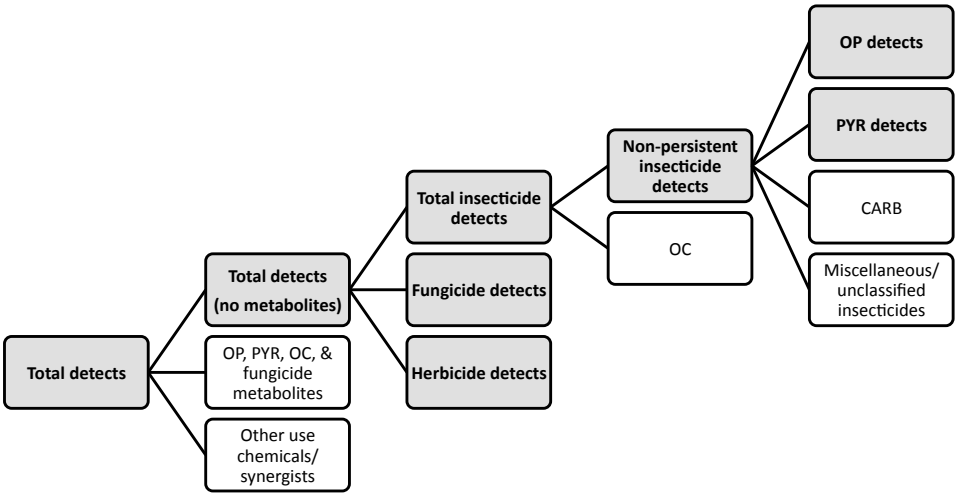
Zhang, M., Fang, X., Zhou, L., Su, L., Zheng, J., Jin, M., et al. (2013). Pesticide poisoning in Zhejiang, China: a retrospective analysis of adult cases registration by occupational disease surveillance and reporting systems from 2006 to 2010. *BMJ Open, 3*(11), e003510.

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*(2), 125-144.

Zhang, Y., Han, S., Liang, D., Shi, X., Wang, F., Liu, W., et al. (2014). Prenatal exposure to organophosphate pesticides and neurobehavioral development of neonates: a birth cohort study in shenyang, china. *PLoS One, 9*(2), e88491.

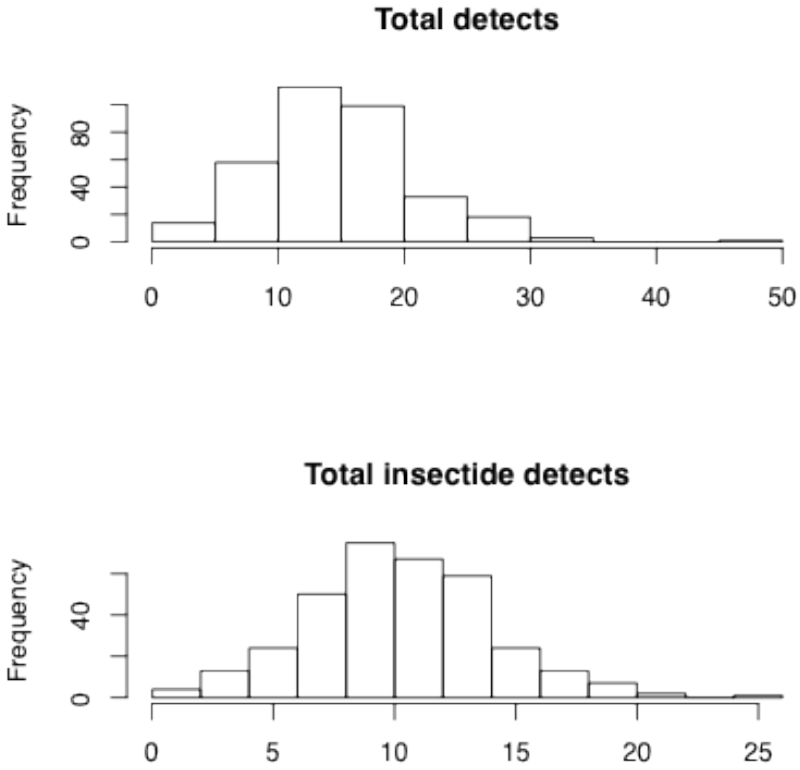
Zhao, G., Xu, Y., Li, W., Han, G., & Ling, B. (2007). Prenatal exposures to persistent organic pollutants as measured in cord blood and meconium from three localities of Zhejiang, China. *Sci Total Environ, 377*(2-3), 179-191.

Figure II.1. Creation of composite pesticide variables [1]



[1-] Analysis variables are shown in shaded boxes

Figure II.2. Distributions of number of detected pesticides and insecticides in cord blood plasma samples of infants from Zhejiang, China (n=336) [1]



[1-] Histograms have different scales

Table II.1. Distribution of pesticide concentrations in umbilical cord blood plasma (ng/mL) at delivery, Zhejiang Province, China (n=336)

Pesticide			Selected Percentiles					
	LOD	n > LOD (%)	50th	75th	90th	95th	99th	Max
Organophosphates (OP)								
Acephate	0.10	13 (3.9)	ND	ND	ND	ND	0.53	0.68
Chlorpyrifos	0.68	136 (40.5)	ND	0.96	3.85	6.24	9.08	11.40
Chlorpyrifos-methyl	0.01	20 (6.0)	ND	ND	ND	0.04	0.37	1.14
Diazinon	0.003	1 (0.3)	ND	ND	ND	ND	ND	0.38
Fensulfothion	0.03	1 (0.3)	ND	ND	ND	ND	ND	10.35
Fosthiazate	0.07	1 (0.3)	ND	ND	ND	ND	ND	7.82
Isofenphos-methyl	0.13	6 (1.8)	ND	ND	ND	ND	0.57	14.70
Methamidophos	1.52	218 (64.9)	4.23	24.54	63.85	115.94	231.96	496.86
Methidathion	0.07	1 (0.3)	ND	ND	ND	ND	ND	9.23
Mevinphos	0.12	27 (8.0)	ND	ND	ND	0.20	2.25	4.03
Monocrotophos	0.01	1 (0.3)	ND	ND	ND	ND	ND	0.05
Naled	0.42	312 (92.9)	1.77	5.14	11.06	20.03	50.31	74.68
Omethoate	1.35	116 (34.5)	ND	12.38	44.49	63.10	106.64	213.22
Phorate	1.79	103 (30.7)	ND	2.77	7.81	11.10	25.92	50.13
Terbufos	0.33	5 (1.5)	ND	ND	ND	ND	0.87	3.32
Trichlorfon	0.35	189 (56.3)	0.73	2.84	9.74	19.61	35.75	43.25
Carbophenothion sulfone [m]	0.02	43 (12.8)	ND	ND	0.22	0.55	1.64	18.83
DEDTP [m]	0.06	99 (29.5)	ND	0.43	1.09	1.55	2.44	3.63
DMDTP [m]	1.74	21 (6.3)	ND	ND	ND	2.12	4.87	21.93
DMTP [m]	1.35	1 (0.3)	ND	ND	ND	ND	ND	9.24
Phorate sulfone [m]	0.01	6 (1.8)	ND	ND	ND	ND	0.04	1.19
TCPY [m]	2.32	2 (0.6)	ND	ND	ND	ND	ND	13.52

Abbreviations: ND, non-detect; DEDTP, Diethyldithiophosphate; DMDTP, Dimethyldithiophosphate; DMTP, Dimethylthiophosphate; TCPY, 3,5,6-trichloro-2-pyridinol

[m] Denotes a metabolite

Table II.1, continued

Pesticide			Selected Percentiles					
	LOD	n > LOD (%)	50th	75th	90th	95th	99th	Max
Pyrethroids (PYR)								
Cyfluthrin	0.84	114 (33.9)	ND	1.49	3.06	4.00	10.55	1158.34
λ-Cyhalothrin	0.51	180 (53.6)	0.75	4.35	8.79	12.76	18.99	24.86
Cypermethrin	3.54	143 (42.6)	ND	7.58	14.53	20.39	35.55	390.27
Etofenprox	5.08	260 (77.4)	30.77	102.47	171.39	226.69	410.10	502.75
Fenpropathrin	0.05	150 (44.6)	ND	0.68	2.13	3.51	12.02	23.25
Fenvalerate	5.06	40 (11.9)	ND	ND	5.59	7.87	13.76	206.13
Flucythrinate	0.06	8 (2.4)	ND	ND	ND	ND	0.60	198.54
Fluvalinate-tau	6.08	46 (13.7)	ND	ND	9.15	15.87	27.02	39.60
Cis-Permethrin	2.19	252 (75.0)	6.92	15.57	28.32	39.28	278.91	470.05
Trans-Permethrin	0.03	239 (71.1)	2.81	124.10	314.95	449.97	596.69	737.84
Tefluthrin	0.17	7 (2.1)	ND	ND	ND	ND	0.21	0.95
Tetramethrin	3.88	59 (17.6)	ND	ND	12.45	20.22	33.74	41.48
3-Phenoxybenzoic acid [m]	0.12	297 (88.4)	4.16	45.70	87.43	115.86	174.02	202.24
Carbamates (CARB)								
Pirimicarb	0.05	2 (0.6)	ND	ND	ND	ND	ND	0.41
Propoxur	0.01	334 (99.4)	0.13	12.73	29.00	35.29	49.44	58.66
Organochlorines (OC)								
Aldrin	0.14	276 (82.1)	1.66	3.90	7.04	11.36	28.16	40.75
Dicofol	2.54	28 (8.3)	ND	ND	ND	3.39	8.94	25.56
Dieldrin	5.57	31 (9.2)	ND	ND	ND	7.87	19.57	30.40
Mirex	0.003	77 (22.9)	ND	ND	0.07	0.16	1.59	120.00
Pentachlorophenol	0.01	22 (6.5)	ND	ND	ND	0.13	1.27	2.19
β-BHC [m]	0.01	15 (4.5)	ND	ND	ND	ND	1.32	12.92
o,p'-DDE [m]	0.04	88 (26.2)	ND	0.08	1.22	2.95	16.70	28.98
p,p'-DDE [m]	0.03	121 (36.0)	ND	31.57	119.49	201.57	381.21	1101.73

Abbreviations: ND, non-detect; β-BHC, β-Hexachlorohexane; o,p'-DDE, *o,p'*-dichlorodiphenyldichloroethylene; p,p'-DDE, *p,p'*-dichlorodiphenyldichloroethylene; [m] Denotes a metabolite

Table II.1, continued

Pesticide			Selected Percentiles					
	LOD	n > LOD (%)	50th	75th	90th	95th	99th	Max
Miscellaneous/ Unclassified Insecticides								
EPN	0.48	7 (2.1)	ND	ND	ND	ND	3.56	16.14
Pyraclofos	0.04	1 (0.3)	ND	ND	ND	ND	ND	13.22
Prothiofos	5.06	149 (44.3)	ND	13.08	30.12	44.98	70.44	104.49
Pyridaben	0.30	26 (7.7)	ND	ND	ND	0.51	1.49	18.54
Spirodiclofen	0.04	3 (0.9)	ND	ND	ND	ND	ND	24.20
Fungicides								
Dicloran	0.003	1 (0.3)	ND	ND	ND	ND	ND	0.05
Difenoconazole	0.07	1 (0.3)	ND	ND	ND	ND	ND	13.40
Dimethomorph	0.01	1 (0.3)	ND	ND	ND	ND	ND	10.95
Furalaxyl	0.03	1 (0.3)	ND	ND	ND	ND	ND	11.13
Metalaxyl	0.01	68 (20.2)	ND	ND	0.22	0.42	0.61	0.66
Myclobutanil	0.07	1 (0.3)	ND	ND	ND	ND	ND	8.52
Nuarimol	0.01	1 (0.3)	ND	ND	ND	ND	ND	11.90
Oxadixyl	0.01	104 (31.0)	ND	0.32	1.25	4.74	17.83	60.07
Paclobutrazole	1.35	1 (0.3)	ND	ND	ND	ND	ND	10.56
Triadimefon	0.07	1 (0.3)	ND	ND	ND	ND	ND	9.76
Triflumizole	0.02	2 (0.6)	ND	ND	ND	ND	ND	8.86
Quinoxyfen	0.34	18 (5.4)	ND	ND	ND	0.35	4.21	35.43
Tetrahydrophthalimide [m]	0.34	61 (18.2)	ND	ND	0.64	0.96	2.56	3.14

Abbreviations: ND, non-detect; EPN, Ethyl *p*-nitrophenyl thionobenzenephosphonate
[m] Denotes a metabolite

Table II.1, continued

Pesticide			Selected Percentiles					
	LOD	n > LOD (%)	50th	75th	90th	95th	99th	Max
Herbicides								
Atrazine	0.01	25 (7.4)	ND	ND	ND	0.01	0.02	0.02
Barban	5.57	1 (0.3)	ND	ND	ND	ND	ND	8.25
Dicamba	1.27	6 (1.8)	ND	ND	ND	ND	1.87	2.74
Diphenamid	0.02	1 (0.3)	ND	ND	ND	ND	ND	9.09
2,4-D	0.51	73 (21.7)	ND	ND	1.26	1.80	4.05	58.24
Diuron	0.04	12 (3.6)	ND	ND	ND	ND	1.25	12.84
Fluridone	1.35	1 (0.3)	ND	ND	ND	ND	ND	10.85
Prometryn	0.02	208 (61.9)	1.20	7.50	17.34	29.30	64.12	182.18
Simazine	0.25	63 (18.8)	ND	ND	0.45	0.71	1.68	2.87
Other-use chemicals/ synergists								
1-Hydroxynaphthalene	1.29	56 (16.7)	ND	ND	1.44	1.84	2.60	3.31
Piperonyl butoxide	0.22	14 (4.2)	ND	ND	ND	ND	1.30	16.57
Triphenylphosphate	0.07	111 (33.0)	ND	0.91	3.48	5.12	19.19	73.00

Abbreviations: ND, non-detect; 2,4-D, 2,4-Dichlorophenoxyacetic acid
[m] Denotes a metabolite
Undetected pesticides: OP- dicrotophos, dimethoate, formothion, phosphamidon, dimethylvinphos, methyl parathion, malathion, dichlorvos; CARB- bendiocarb; FUNG- pyrimethanil, vinclozolin; FUNG metabolite- pentachloroaniline; HERB- dimethipin, monolinuron, clomazone, isocarbamide, propyzamide, terbacil, dimethenamid, metribuzin, alachlor.

Table II.2. Family and household characteristics of the study population (n=237)

Characteristics	N	Mean (SD)	Range
# Family in home	220	5.1 (1.3)	1-11
# People in home	210	4.3 (1.4)	1-9
Living space (square meters)	215	214.1 (147.9)	18-720
Maternal age (years)	216	26.1 (3.9)	18-41
Paternal age (years)	205	28.4 (4.4)	19-47
Characteristics	**N**	**N (%)**	
Place of residence	216		
Rural		141 (65.3)	
City		75 (34.7)	
Annual income	215		
< 30,000 Yuan		42 (19.5)	
30,000-49,999 Yuan		41 (19.1)	
50,000-99,999 Yuan		66 (30.7)	
≥100,000 Yuan		66 (30.7)	
Maternal education	221		
Middle school or less		84 (38.0)	
High school/secondary school		64 (29.0)	
College		73 (33.0)	
Paternal education	209		
Middle school or less		84 (40.2)	
High school/secondary school		57 (27.3)	
College		68 (32.5)	
Maternal occupation	221		
Housewife		91 (41.2)	
Other		130 (58.8)	
Paternal occupation	208		
Professional/Admin.		81 (38.9)	
Manager		32 (15.4)	
Factory worker		30 (14.4)	
Other		65 (31.3)	
Season of birth	237		
Spring (March-May)		62 (26.2)	
Summer (June-Sept.)		91 (38.4)	
Fall/Winter (Oct.-Dec.)		84 (35.4)	

Table II.3. Selected results of generalized linear models for composite pesticide exposure variables, analyzing household, parental, and seasonal characteristics as predictors of exposure

Predictor (Referent)	Total detects β (95% CI)	Total detects (no metab.) β (95% CI)	Total insecticide detects β (95% CI)	Non-persistent insecticide detects β (95% CI)
Paternal education (College)				
Middle school or less	-0.05 (-1.66, 1.57)	-0.06 (-1.17, 1.29)	0.07 (-0.92, 1.06)	0.04 (-0.86, 0.93)
High school/ secondary school	-0.58 (-2.38, 1.21)	-0.28 (-1.65, 1.09)	-0.00 (-1.10, 1.11)	-0.05 (-0.95, 1.04)
Maternal occupation (Housewife)				
Other	-0.84 (-2.18, 0.49)	-0.73 (-1.75, 0.29)	-0.66 (-1.48, 0.16)	-0.66 † (-1.40, 0.08)
Season of birth (Fall/Winter)				
Spring	0.64 (-1.02, 2.30)	0.77 (-0.50, 2.04)	0.65 (-0.38, 1.67)	0.63 (-0.30, 1.55)
Summer	2.20 * (0.52, 3.88)	1.59 * (0.31, 2.88)	1.19 * (0.15, 2.22)	1.13 * (0.19, 2.07)
Month of birth (December)				
March	-3.00 (-7.69, 1.69)	-1.93 (-5.52, 1.65)	-1.70 (-4.58, 1.18)	-1.42 (-4.02, 1.17)
April	0.44 (-2.70, 3.58)	0.51 (-1.90, 2.91)	-0.09 (-2.02, 1.84)	-0.07 (-1.80, 1.67)
May	-0.45 (-3.73, 2.83)	-0.27 (-2.78, 2.23)	-0.37 (-2.37, 1.64)	-0.48 (-2.28, 1.34)
June	1.14 (-2.30, 4.59)	0.70 (-1.94, 3.33)	0.11 (-2.00, 2.23)	0.25 (-1.66, 2.16)
July	4.04 * (0.42, 7.65)	3.00 * (0.24, 5.76)	2.15 † (-0.07, 4.37)	2.09 (0.09, 4.09) *
August	1.31 (-2.34, 4.95)	0.74 (-2.04, 3.53)	0.12 (-2.12, 2.35)	-0.15 (-2.16, 1.87)
September	-0.38 (-3.85, 3.08)	-0.63 (-3.28, 2.02)	-1.12 (-3.25, 1.01)	-1.16 (-3.08, 0.75)
October	-1.47 (-4.90, 1.96)	-1.08 (-3.71, 1.54)	-1.39 (-3.50, 0.72)	-1.25 (-3.15, 0.65)
November	-0.52 (-4.00, 2.97)	-0.79 (-3.45, 1.87)	-1.03 (-3.17, 1.11)	-1.14 (-3.07, 0.78)

** = $p < 0.01$, * = $p < 0.05$, † = $p < 0.10$
Additional results can be found in Table II.A1 (Appendix)

Table II.3, continued

Predictor (Referent)	OP detects β (95% CI)	PYR detects β (95% CI)	Fungicide detects β (95% CI)	Herbicide detects β (95% CI)
Paternal education (College)				
Middle school or less	0.03 (-0.43, 0.48)	-0.03 (-0.58, 0.52)	-0.01 (-0.27, 0.24)	0.01 (-0.22, 0.23)
High school/ secondary school	-0.04 (-0.55, 0.46)	0.01 (-0.60-0.62)	-0.29 * (-0.57, -0.01)	0.01 (-0.25, 0.26)
Maternal occupation (Housewife)				
Other	-0.33 † (-0.71, 0.05)	-0.28 (-0.74, 0.19)	0.00 (-0.21, 0.22)	-0.07 (-0.26, 0.12)
Season of birth (Fall/Winter)				
Spring	0.00 (-0.48, 0.47)	0.40 (-0.17, 0.98)	-0.12 (-0.38, 0.15)	0.24 * (0.01, 0.48)
Summer	0.41 † (-0.07, 0.88)	0.63 * (0.04, 1.21)	0.17 (-0.10, 0.44)	0.24 † (0.00, 0.47)
Month of birth (December)				
March	-1.31 † (-2.65, 0.03)	0.06 (-1.55, 1.66)	-0.46 (-1.21, 0.29)	0.22 (-0.44, 0.88)
April	-0.14 (-1.04, 0.76)	0.01 (-1.06, 1.09)	0.02 (-0.49, 0.52)	0.58 * (0.14, 1.02)
May	-0.45 (-1.39, 0.49)	0.05 (-1.08, 1.17)	-0.19 (-0.71, 0.34)	0.28 (-0.18, 0.74)
June	-0.10 (-1.08, 0.89)	0.44 (-0.74, 1.62)	0.07 (-0.48, 0.62)	0.51 * (0.02, 0.99)
July	0.57 (-0.46, 1.61)	1.52 * (0.28, 2.76)	0.11 (-0.47, 0.69)	0.74 ** (0.23, 1.25)
August	0.00 (-1.03, 1.05)	0.06 (-1.19, 1.30)	0.33 (-0.25, 0.91)	0.30 (-0.21, 0.81)
September	-0.14 (-1.13, 0.85)	-0.80 (-1.98, 0.39)	0.27 (-0.29, 0.82)	0.22 (-0.27, 0.71)
October	-0.69 (-1.68, 0.29)	-0.25 (-1.42, 0.92)	-0.14 (-0.69, 0.41)	0.44 † (-0.38, 0.93)
November	-0.16 (-1.15, 0.84)	-0.72 (-1.91, 0.48)	0.20 (-0.36, 0.76)	0.04 (-0.45, 0.53)

** = $p < 0.01$, * = $p < 0.05$, † = $p < 0.10$
Additional results can be found in Table II.A1 (Appendix)

Table II.4. Significant results of generalized linear models for individual pesticides with ≥80% detection rate and logistic regression models for pesticides with detection rates of 50-80%, analyzing household, parental, and seasonal characteristics as predictors of exposure

Predictor (Referent)	Continuous Pesticide Results	Dichotomous Pesticide Results
	β (95% CI) [1]	OR (95% CI) [2]
Maternal age	3-PBA: 1.20 (0.05, 2.34) *	
Maternal occupation (Housewife)		
Other		Methamidophos: 1.92 (1.20, 3.08)
Season of birth (Fall/Winter)		
Spring	3-PBA: -21.54 (-33.50, -9.60) *** Propoxur: -7.19 (-10.62, -3.76) *** Aldrin: 1.37 (-0.16, 2.89) †	Prometryn: 0.44 (0.25, 0.78)
Summer	Naled: 4.17 (1.42, 6.92) **	Trichlorfon: 0.55 (0.31, 0.95) *Trans*-permethrin: 0.49 (0.26, 0.91)
Month of birth (December)		
March	Propoxur: -8.08 (-17.50, 1.35) †	
June	Aldrin: 3.07 (0.10, 6.24) †	
July	Naled: 8.81 (3.13, 14.49) **	
September	3-PBA: 22.73 (-1.62, 47.08) † Propoxur: 5.84 (-1.12, 12.81) †	
November	3-PBA: 25.95 (1.45, 50.44) * Propoxur: 8.03 (0.99, 15.07) *	Prothiophos: 3.91 (1.15, 13.28)

[1] *** p < 0.001, ** p < 0.01, * p < 0.05, † p < 0.10
[2] Modeled the probability that pesticide <LOD, so a value <1 means higher odds of detection, while a value >1 means lower odds of detection.
Abbreviations: 3-PBA, 3- Phenoxybenzoic Acid; OR, odds ratio
Additional results can be found in Table II.A2 (Appendix)

Table II.5. Comparison of cord blood serum or plasma samples from the current study and previously published studies in the U.S. and China (ng/mL)

	Current study [1]	U.S. studies [2]	China studies [1]
Organophosphates (OP)			
Chlorpyrifos	3.85	0.014 [d] 0.002 [g] 0.065 [e]*	0.17 [f]
Diazinon	0.38 max	ND [d] 0.003 [g] 0.013 [e]*	0.27 [f]
Dichlorvos	ND	0.005 [e]*	NM
Malathion	ND	0.048 [e]*	3.13 [f]
Methyl parathion	ND	0.016 [e]*	1.43 [f]
Phorate	7.81	0.010 [e]*	NM
Terbufos	3.32 max	0.071 [e]*	0.27 [f]
Pyrethroids (PYR)			
Cyfluthrin	3.06	0.084 [d]	NM
Cyhalothrin	8.79	ND [d]	NM
Cypermethrin	14.53	ND [d]	NM
Fenvalerate	5.59	ND [d]	NM
Cis-Permethrin	28.32	0.010 [d] 0.001 [g] 0.004 [e]*	NM
Trans-Permethrin	314.95	0.028 [d] 0.002 [g] 0.005 [e]*	NM
Tetramethrin	12.45	ND [d]	NM
Carbamates (CARB)			
Bendiocarb	ND	0.032 [e]*	NM
Propoxur	29.00	0.033 [d] 0.670 [e]*	0.19 [f]
Organochlorines (OC)			
Aldrin	7.04	NM	5.56 max, [a]
Dieldrin	7.87 95th	NM	20.79 max, [a]
Mirex	0.07	0.03 [d]	0.16 max, [b]*
β-BHC [m]	12.92 max	NM	9.69 max, [a] 0.09 med, [c]* 1.79 max, [b]* 0.07, 0.33, 0.14 max, [h]*
o,p'-DDE [m]	1.22	NM	0.03 max, [b] ND med, [c]*
p,p'-DDE [m]	119.49	17.73 [d]	31.66 max, [a] 1.32 med, [c]* 17.16 max, [b]* 1.37, 9.76, 85.19 max, [h]*
Fungicides (FUNG)			
Dicloran	0.05 max	0.03 [e]*	4.73 [f]
Metalaxyl	0.22	0.02 [g] 0.26 [e]*	18.60 max, [f]
Vinclozolin	ND	NM	0.94 [f]
Tetrahydrophthalimide [m]	0.64	0.01 [g] 0.04 [e]*	
Herbicides (HERB)			
Alachlor	ND	0.02 [e]*	2.21 [f]
Atrazine	0.01 95th	0.01 [e]*	1.47 [f]
Non-pesticide/synergist			
Piperonyl butoxide	16.57 max	0.0001 [e]	NM

Abbreviations: ND, non-detect; NM, not measured.
[m] Denotes a metabolite.
[1] 90th percentile concentrations are shown unless otherwise indicated.
[2] Maximum concentrations are shown unless otherwise indicated.
[max] Maximum; [95th] 95th percentile; [90th] 90th percentile; [med] Median.
[a] Cao, 2011; [b] Guo, 2014; [c] Li, 2014; [d] Neta, 2010; [e] Whyatt, 2013; [f] Wickerham, 2012; [g] Yan, 2009; [h] Zhao, 2007
* Denotes an estimated value: [e]- data estimated by converting from pg/g plasma to ng/mL by multiplying by 1.03/1000 (weight of plasma is 1.03 g/mL; there are 1000 pg per ng); [b, c, h]- data estimated by converting from ng/g lipid to ng/mL (non-lipid adjusted) by multiplying by 6.84/1000 (calculated average of lipid concentration as 6.84 g lipid/L**; there are 1000 ml per L).
**Reported levels of triglyceride (TG)= 3.02 mmol/L and cholesterol, LDL = 3.07 mmol/L and HDL= 1.76 mmol/L in Hangzhou infant cord blood (Hou, et al., 2014). We converted these values to mg/dl and determined total lipids using the equation: total lipid (g/L) = 0.9 + 1.3(chol (g/L) + TG (g/L)) (Li, et al., 2014; Rylander, Nilsson-Ehle, & Hagmar, 2006). Using this equation we estimated a total lipid concentration in Chinese infants to be 6.84 g lipid/L.

Chapter II Appendix

Table II.A1. Additional results of generalized linear models for composite pesticide exposure variab1.25les, analyzing household, parental, and seasonal characteristics as predictors of exposure

Predictor (Referent)	Total detects β (95% CI)	Total detects (no metabolites) β (95% CI)	Total insecticide detects β (95% CI)	Total insecticide detects (no OCs) β (95% CI)
# Family in home	-0.12 (-0.63, 0.39)	-0.05 (-0.44, 0.33)	-0.07 (-0.39, 0.24)	-0.08 (-0.36, 0.20)
# People in home	0.04 (-0.42, 0.50)	0.05 (-0.31, 0.40)	0.02 (-0.26, 0.31)	0.00 (-0.26, 0.26)
Living space	0.00 (-0.01, 0.00)	0.00 (-0.00, 0.00)	0.00 (-0.00, 0.00)	0.00 (-0.00, 0.00)
Residence (Urban)				
Rural	-0.06 (-1.47, 1.35)	0.04 (-1.03, 1.12)	0.01 (-0.86, 0.87)	0.03 (-0.75, 0.81)
Income (≥100,000 Yuan)				
< 30,000 Yuan	-0.49 (-2.42, 1.44)	-0.46 (-1.93, 1.01)	-0.21 (-1.39, 0.97)	-0.15 (-1.22, 0.91)
30,000-49,999 Yuan	0.44 (-1.59, 2.47)	0.07 (-1.48, 1.61)	0.23 (-1.01, 1.47)	0.08 (-1.04, 1.19)
50,000-99,999 Yuan	0.28 (-1.59, 2.46)	0.15 (-1.26, 1.56)	0.06 (-1.07, 1.20)	0.02 (-1.00, 1.04)
Maternal age	0.05 (-0.13, 0.24)	0.02 (-0.13, 0.16)	0.00 (-0.11, 0.12)	-0.01 (-0.12, 0.09)
Paternal age	-0.06 (-0.22, 0.10)	-0.05 (-0.18, 0.07)	-0.04 (-0.14, 0.05)	-0.03 (-0.12, 0.05)
Maternal education (College)				
Middle school or less	0.14 (-1.42, 1.70)	0.14 (-1.06, 1.33)	0.26 (-0.70, 1.22)	0.25 (-0.62, 1.11)
High school/ Secondary school	0.12 (-1.58, 1.82)	0.32 (-0.98, 1.62)	0.37 (-0.67, 1.41)	0.42 (-0.53, 1.36)
Paternal occupation (Prof./Tech./Admin.)				
Manager	0.21 (-1.77, 2.19)	0.04 (-1.47, 1.56)	-0.15 (-1.36, 1.07)	-0.13 (-1.23, 0.97)
Factory worker	0.50 (-1.44, 2.45)	0.31 (-1.18, 1.80)	0.15 (-1.05, 1.34)	0.02 (-1.06, 1.10)
Other	-0.09 (-1.81, 1.62)	-0.01 (-1.31, 1.30)	-0.09 (-1.14, 0.96)	-0.25 (-1.20, 0.70)

Table II.A1, continued

Predictor (Referent)	OP detects β (95% CI)	PYR detects β (95% CI)	Fungicide detects β (95% CI)	Herbicide detects β (95% CI)
# Family in home	0.03 (-0.12, 0.17)	-0.12 (-0.30, 0.05)	-0.02 (-0.10, 0.06)	0.04 (-0.04, 0.11)
# People in home	0.08 (-0.05, 0.21)	-0.08 (-0.24, 0.08)	0.02 (-0.05, 0.10)	0.00 (-0.07, 0.07)
Living space	0.00 (-0.00, 0.00)	0.00 (-0.00, 0.00)	0.00 (-0.00, 0.00)	0.00 (-0.00, 0.00)
Residence (Urban)				
Rural	-0.09 (-0.49, 0.31)	0.10 (-0.39, 0.58)	-0.01 (-0.24, 0.21)	0.08 (-0.12, 0.27)
Income (≥100,000 Yuan)				
< 30,000 Yuan	-0.01 (-0.55, 0.54)	-0.15 (-0.81, 0.52)	-0.04 (-0.34, 0.27)	-0.21 (-0.49, 0.06)
30,000-49,999 Yuan	0.26 (-0.31, 0.84)	-0.27 (-0.97, 0.43)	0.03 (-0.30, 0.35)	-0.19 (-0.47, 0.10)
50,000-99,999 Yuan	-0.08 (-0.61, 0.44)	0.01 (-0.65, 0.63)	0.17 (-0.13, 0.47)	-0.08 (-0.34, 0.18)
Maternal age	0.02 (-0.04, 0.07)	-0.02 (-0.09, 0.04)	0.02 (-0.01, 0.05)	-0.01 (-0.04, 0.02)
Paternal age	0.00 (-0.05, 0.04)	-0.02 (-0.07, 0.03)	-0.00 (-0.03, 0.02)	-0.01 (-0.03, 0.02)
Maternal education (College)				
Middle school or less	0.04 (-0.40, 0.48)	0.13 (-0.41, 0.66)	-0.03 (-0.27, 0.22)	-0.10 (-0.32, 0.12)
High school/ Secondary school	0.20 (-0.29, 0.68)	0.12 (-0.47, 0.70)	-0.05 (-0.32, 0.22)	0.00 (-0.24, 0.24)
Paternal occupation (Prof./Tech./Admin.)				
Manager	-0.06 (-0.63, 0.49)	-0.03 (-0.71, 0.65)	0.03 (-0.28, 0.35)	0.16 (-0.12, 0.43)
Factory worker	-0.05 (-0.61, 0.50)	0.10 (-0.57, 0.76)	0.09 (-0.22, 0.40)	0.08 (-0.20, 0.35)
Other	-0.23 (-0.72, 0.25)	-0.03 (-0.61, 0.56)	0.08 (-0.19, 0.36)	0.00 (-0.24, 0.24)

Table II.A2. Significant results of logistic regression models for pesticides with detection rates of 10-50%, analyzing household, parental, and seasonal characteristics as predictors of exposure

Predictor (Referent)	Dichotomous Pesticide Results OR (95% CI) [1]
# People in home	Mirex: 0.83 (0.70, 0.99)
Income (≥100,000 Yuan)	
30,000-49,999 Yuan	Omethoate: 0.44 (0.22, 0.90)
Maternal age	DEDTP: 0.92 (0.86-0.98) Carbophenothion sulfone: 0.91 (0.84, 0.99) Mirex: 0.92 (0.86, 0.99) Metalaxyl: 0.92 (0.86, 0.99)
Paternal age	o,p'-DDE: 1.08 (1.01, 1.15)
Maternal education (College)	
Middle school or less	2,4-D: 2.02 (1.09, 3.75)
Paternal education (College)	
High school/ secondary school	Carbophenothion sulfone: 0.40 (0.16, 0.99) Oxadixyl: 2.36 (1.22, 4.56)
Maternal occupation (Housewife)	
Other	Fluvalinate-tau: 2.07 (1.10, 3.90) Tetramethrin: 1.78 (1.01, 3.14)
Season of birth (Fall/Winter)	
Spring	Omethoate: 2.55 (1.37, 4.74) Chlorpyrifos: 0.41 (0.22, 0.77) DEDTP: 2.55 (1.37, 4.74) Cypermethrin: 0.36 (0.20, 0.65) Fenvalerate: 0.32 (0.10, 0.98) Tetramethrin: 2.95 (1.42, 6.13) o,p'-DDE: 0.43 (0.22, 0.85) p,p'-DDE: 2.07 (1.16, 3.69) Prothiophos: 0.47 (0.27, 0.83)
Summer	Chlorpyrifos: 0.25 (0.13, 0.46) Fenvalerate: 0.26 (0.09, 0.80) o,p'-DDE: 0.48 (0.24, 0.95) Tetrahydrophthalimide: 0.45 (0.21, 0.95)
Month of birth (December)	
March	Cypermethrin: 0.12 (0.02, 0.71)
April	Cypermethrin: 0.23 (0.07, 0.76) Tetramethrin: 6.50 (1.71, 24.75)
June	Chlorpyrifos: 0.15 (0.04, 0.55) Tetramethrin: 8.25 (1.46, 46.60) o,p'-DDE: 0.24 (0.07, 0.88)
July	Chlorpyrifos: 0.10 (0.03, 0.41) 2,4-D: 0.16 (0.03, 0.82)

[1] = Modeled the probability that pesticide <LOD, so a value <1 means higher odds of detection, while a value >1 means lower odds of detection.
Abbreviations: 2,4-D, 2,4-Dichlorophenoxyacetic acid

CHAPTER III

AIM 2: Prenatal exposure to organophosphate insecticides and infant motor function

Abstract

Background: Organophosphate insecticides (OPs) are used worldwide, yet despite nearly ubiquitous exposure in the general population, few have been studied outside the laboratory. Fetal brains undergo rapid growth and development, leaving them susceptible to long-term effects of neurotoxic OPs. The objective of this study was to investigate the extent to which prenatal exposure to OPs affects infant motor development.

Methods: 30 OPs were measured in umbilical cord blood using gas chromatography tandem mass spectrometry in a cohort of Chinese infants. Motor function was assessed at 6-weeks and 9-months using Peabody Developmental Motor Scales 2nd edition (PDMS-2) (n=199). Outcomes included subtest scores: reflexes, stationary, locomotion, grasping, visual-motor integration (V-M), composite scores: gross (GM), fine (FM), total motor (TM), and standardized motor quotients: gross (GMQ), fine (FMQ), total motor (TMQ). Neuromotor function was assessed using a secondary test, the Infant Neurological International Battery (INFANIB). INFANIB outcomes included subscales for

spasticity/muscle tone, head and trunk control, vestibular function, legs/lower limb function, French angles, and total score.

Results: Naled, methamidophos, trichlorfon, chlorpyrifos, and phorate were detected in ≥10% of samples. Prenatal naled and chlorpyrifos were associated with decreased 9-month motor function. Scores were 0.55, 0.85, and 0.90 points lower per 1 ng/mL increase in log-naled, for V-M (p=0.04), FM (p=0.04), and FMQ (p=0.08), respectively. For chlorpyrifos, scores were 0.50, 1.98, 0.80, 1.91, 3.49, 2.71, 6.29, 2.56, 2.04, and 2.59 points lower for exposed versus unexposed infants, for reflexes (p= 0.04), locomotion (p=0.02), grasping (p=0.05), V-M (p<0.001), GM (p=0.007), FM (p=0.002), TM (p<0.001), GMQ (p=0.01), FMQ (p=0.07), and TMQ (p=0.008), respectively. INFANIB analyses did not yield any significant associations, however general trends indicate reduced INFANIB scores at 6 weeks for infants with higher prenatal OP exposures. Girls appeared to be more sensitive to the negative effects of OPs on 9-month motor function than boys.

Conclusions: We found deficits in 9-month motor function in infants with prenatal exposure to naled and chlorpyrifos. Naled is being aerially sprayed to combat mosquitoes carrying Zika virus, yet this is the first non-occupational human study of its health effects. Delays in early-motor skill acquisition may be detrimental for downstream development and cognition.

Introduction

Synthetic pesticides are used extensively for pest management in a wide range of residential, occupational, and agricultural settings. China reports some of the highest pesticide usage rates in the world (Ding & Bao, 2013; U.S. EPA, 2011; W. Zhang, Jiang, & JF., 2011), at up to 5 times the global average (Huang, Qiao, Zhang, & Rozelle, 2001; Y. Zhang, et al., 2014). Organophosphate insecticides (OPs) account for more than a third of all insecticide use in China (Y. Zhang, et al., 2014). The primary route of OP exposure in the general population is via the diet, though exposure can also occur from ingestion of contaminated drinking water or dust, residential pest control applications, or topical treatments (CDC, 2016; Huang, et al., 2001; NPIC, 2010). Additionally, warming temperatures have seen a surge in the transmission of mosquito-borne infectious diseases (Bai, Morton, & Liu, 2013), likely leading to aerial OP spraying to combat disease spread.

OPs are neurotoxicants, and over the last couple of decades have emerged as a particular concern for developmental neurotoxicity. Developing fetal brains undergo rapid growth and maturation, leaving them susceptible to possible long-term effects of exposure (Garcia, Seidler, & Slotkin, 2005). Associations have been reported between prenatal exposures to OPs and deficits in IQ (Bouchard, et al., 2011; Engel, et al., 2011; V. Rauh, et al., 2011), and increases in autism spectrum (Shelton, et al., 2014), attention deficit-hyperactivity (Marks, et al., 2010; V. A. Rauh, et al., 2006), and pervasive developmental disorder (Eskenazi, et al., 2007; V. A. Rauh, et al., 2006).

Despite a growing body of evidence regarding prenatal OP exposure and such neurodevelopmental endpoints, less is known about effects on early-life motor function. Motor skill acquisition in infancy provides a foundation for downstream cognitive and socio-emotional development in childhood (Clearfield, 2004, 2011). Motor functions improve rapidly in infancy with increasing central nervous system maturation and serve as an early benchmark of healthy neurological development (Noritz & Murphy, 2013). Delays in meeting early motor milestones may be indicative of a developmental disorder (De Felice, Scattoni, Ricceri, & Calamandrei, 2015; Noritz & Murphy, 2013).

Epidemiological studies provide preliminary evidence that prenatal OP exposure may negatively affect infant or child motor function. Maternal urinary OP metabolites during pregnancy (total dialkyl phosphates [DAPs] (Young, et al., 2005; Y. Zhang, et al., 2014), dimethylphosphates [DMPs] (Young, et al., 2005), diethylphosphates [DEPs] (Engel, et al., 2007; Young, et al., 2005), and malathion dicarboxylic acid [MDA] (Engel, et al., 2007)) have been associated with deficits in infant/newborn reflexes. Chlorpyrifos, measured directly in umbilical cord plasma, has been found to be inversely associated with psychomotor development in 3-year-olds (V. A. Rauh, et al., 2006). Two studies of maternal occupational exposure to unspecified OPs during pregnancy found deficits in fine, but not gross, motor skills in infants (Handal, Harlow, Breilh, & Lozoff, 2008) and reduced motor speed and coordination in 6- to 8-year-olds (Harari, et al., 2010).

The current study sought to investigate the effects of prenatal OP exposure, measured directly in cord blood, on infant motor function.

Methods

Pregnant women with healthy, uncomplicated, singleton pregnancies (n=359) were recruited at 37-42 weeks gestation from Fuyang Maternal and Children's hospital between 2008 and 2011 and enrolled into a longitudinal study of iron deficiency and infant neurodevelopment. Of the 359 participants, 237 had a sufficient volume of cord blood for pesticide analysis. Written informed consent was obtained, and the institutional review boards of the University of Michigan and Zhejiang University Children's Hospital approved this study.

Cord blood pesticides

The protocol for the determination of pesticides in cord blood has been described elsewhere (Silver, et al., 2016). Briefly, cord blood plasma samples were at analyzed the Institute of Toxicology at Nanjing Medical University using gas chromatography tandem mass spectrometry (GC-MS/MS). Methods were modified from previously published protocols (Perez, et al., 2010; ThermoScientific). We analyzed for 24 organophosphate (OP) insecticides and 6 OP metabolites. Limits of detection (LODs) were determined by analyzing fortified serum on a signal-to-noise (S/N) ratio of three. Quality control samples were generated using serum samples with 0.675 and 1.35 ng/mL pesticide

standards. Quality control samples and blanks were analyzed in parallel with study samples in each batch.

Individual OPs were treated as continuous when detection rates were ≥80% (values below the limit of detection [<LOD] were replaced with LOD/√2), three-level ordinal (<LOD/medium/high [median split for those above LOD]) when detection rates were 40-79%, and dichotomous (<LOD/detect) when detection rates were 10-39%. Naled (99.6% detected) was log-transformed prior to statistical analysis to account for a right-skewed distribution. Methamidophos and trichlorfon (64.6% and 51.0% detected) were converted to 3-level ordinal variables, while chlorpyrifos and phorate (36.7% and 16.9% detected) were treated as dichotomous. A "number of OP detects" variable was created by assigning OP measurements <LOD a value of 0, while detects were assigned a value of 1; these were summed to create an index of OP exposure for each infant (Wickerham, et al., 2012).

Peabody Developmental Motor Scales 2nd edition (PDMS-2)

Peabody Developmental Motor Scales (PDMS-2) (Folio & Fewell, 1983, 2000) was used as the primary test of motor function in this study. PDMS-2 is a standardized test that assesses gross and fine motor abilities in children from birth through 5 years. PDMS-2 was administered here around 6 weeks and 9 months of age. The PDMS-2 has been proven to have excellent internal consistency (r = 0.89-0.97), test-retest reliability (r = 0.89-0.96), and inter-rater reliability (r =0.96-0.99) (Folio & Fewell, 2000). For this study, PDMS-2 testing was performed by four examiners, with one serving as reference. After training,

agreement between the reference and the other three examiners was 95% or higher. Inter- and intra-tester reliability measures were also monitored over the course of the study.

The gross motor function assessment is comprised of 4 multi-item subtests (reflexes, stationary, and locomotion) that measure interrelated motor abilities of large muscle systems (Folio & Fewell, 1983, 2000). Gross motor subtest scores were summed to create a composite gross motor raw score (GM). The fine motor function assessment is comprised of two multi-item subtests (grasping and visual-motor integration [V-M]) that measure the development of fine muscle systems. Fine motor subtest scores were summed to create a composite fine motor raw score (FM). GM and FM were summed to create a composite total motor raw score (TM) to measure overall motor abilities. Additionally, raw subtest scores were converted to standard scores using PDMS-2 guidelines. Standard scores were then summed and converted to gross (GMQ), fine (FMQ), and total motor quotients (TMQ) according to PDMS-2 guidelines (Folio & Fewell, 1983). PDMS-2 data was available for 199 infants.

Infant Neurological International Battery (INFANIB)

The Infant Neurological International Battery (INFANIB) (Ellison, 1986; Ellison, Horn, & Browning, 1985) was used as a secondary test of motor function. INFANIB is a neuromotor examination of reflexes, joints angles, and posture in infants from birth through 18 months of age. For this study, INFANIB was assessed at 6 weeks and 9 months. The test is comprised of 20 items, each of which are scored 1 to 5, where 1 is severely abnormal and 5 is normal (Ellison,

1986; Ellison, et al., 1985). The subtests include: spasticity/muscle tone (4 items), head and trunk control (4 items), vestibular function (4 items), legs/lower limb function (4 items), and French angles (shoulder and hip angles) (4 items). Subscale scores were summed to create a total score of overall neurological integrity. We examined INFANIB outcomes as raw individual subscale scores and total score. INFANIB data was available for 197 infants.

Statistical analysis

Statistical analyses were conducted using SAS 9.3 (Cary, North Carolina). Percentile tables were created to determine the individual OP distributions within the sample. Descriptive statistics and frequencies were examined for all covariates of interest, including sex, age at motor testing, cord ferritin, gestational age, birth weight, maternal education and occupation, family income, and season of motor testing.

Linear mixed models (LMM) with random intercepts were used to evaluate associations between cord OP exposures and PDMS raw scores (subtest [reflexes, stationary, locomotion, grasping, V-M] and composite scores [GM, FM, TM]), as well as motor quotients (GMQ, FMQ, TMQ), at 6 weeks and 9 months. LMM was also used to evaluate associations between cord OP exposures and INFANIB raw scores (subscales [spasticity/muscle tone, head and trunk control, legs/lower limb function, and French angles] and total score), at 6 weeks and 9 months. Vestibular function was only assessed at 9 months so it was analyzed using generalized linear models (GLM). A number of covariates, including maternal education and occupation, income, and season in which motor testing

took place, were considered, but ultimately excluded, from the final models. We previously reported that income and education were not associated with OP exposure in our sample, while season and naled and maternal occupation and methamidophos were significantly associated (Silver, et al., 2016). Of these factors, only season of testing was associated with both OP exposure and motor outcomes. Inclusion of season in the models did not significantly influence the results, therefore the most parsimonious model was chosen to maximize sample size. Final models were adjusted for sex, age at testing, and cord ferritin. To enable comparisons of effect estimates at both of the time points, we included "time" as a class variable and "time*OP" in our LMM models. We used the "lsmeans" procedure to compare estimates for the categorical OP exposures. For continuous exposures (number of OP detects, log-naled), the parameter of interest was the slope estimating change in 6-week or 9-month motor scores per 1 unit increase in OP. For categorical exposures (methamidophos, trichlorfon, chlorpyrifos, phorate), the parameter of interest was the difference in mean 6-week or 9-month motor score by category of OP exposure. To investigate differences in the effect of prenatal OP exposure on infant motor function by sex, we carried out LMM modeling stratified by infant sex. Finally, we determined the odds of an "abnormal" motor outcome at 6 weeks or 9 months, following prenatal OP exposure, using logistic regression. An "abnormal" PDMS score was defined, according to PDMS guidelines, as a subtest standard score or a motor quotient in the lowest 25th percentile. An "abnormal" INFANIB score was defined, according to INFANIB guidelines, as a total score below 66 for 6-week-olds or 83

for 9-month-olds. Logistic regression was only completed if at least 10% of infants had scores that could be defined as “abnormal”. Logistic regression models were adjusted for sex, age at testing, and cord ferritin.

Results

Infants were born to term and of healthy birth weight. Pertinent sample characteristics are presented in Table III.1. Additional household and parental characteristics of the study sample have been previously published (Silver, et al., 2016). Levels of OP exposure for those with and without PDMS data are compared in Appendix Table III.A1. There were no statistically significant differences in exposure between the two groups. Of the 30 OPs and OP metabolites, 18 were detectable in at least one cord blood sample. The mean (standard deviation) number of OPs detected per sample was 2.9 (1.6) and ranged from 1 to 8. Distributions of detectable OPs and their LODs are shown in Table III.2. There were no underlying differences in cord blood pesticide levels by infant sex (Table III.A2).

There were no significant associations between any of the OPs measured and PDMS outcomes at 6 weeks (Appendix Table III.A3). Adjusted LMM results for PDMS outcomes at 9 months are shown in Table III.3. Log-naled was associated with deficits in fine motor function (Table III.3). In adjusted analyses, 9-month raw scores were 0.55 and 0.85 points lower per 1 ng/mL increase in log-naled for V-M ($p=0.04$) and FM ($p=0.04$), respectively. 9-month FMQs were 0.90

points lower per 1 ng/mL increase in log-naled (p=0.08). High prenatal methamidophos exposure (compared to ND) was consistently associated with deficits in PDMS outcomes, though results were not statistically significant. Detectable chlorpyrifos was associated with lower scores for all PDMS subtest and composite scores at 9 months of age (Table III.3). In adjusted analyses, 9-month raw scores were 0.50, 1.98, 0.80, 1.91, 3.49, 2.71, and 6.29 points lower for chlorpyrifos-exposed versus unexposed infants, for reflexes (p= 0.04), locomotion (p=0.02), grasping (p=0.05), V-M (p<0.001), GM (p=0.007), FM (p=0.002), and TM (p<0.001), respectively. 9-month motor quotients were also 2.56, 2.04, and 2.59 points lower in chlorpyrifos-exposed versus unexposed infants, for GMQ (p=0.01), FMQ (p=0.07), and TMQ (p=0.008), respectively.

No statistically significant associations were observed between prenatal OP exposure and infant INFANIB scores at either time point (Table III.4 and Table III.A4 for 6-weeks and 9-months, respectively). However, 6-week subscale and total INFANIB scores were generally lower in infants with higher prenatal OP exposure, compared to unexposed infants (Table III.4).

Bivariate analyses did not revealed any underlying differences in PDMS scores by infant sex (Table III.A5). Sex-stratified LMM results for OP/PDMS associations are shown in Table III.A6 (6 weeks) and Figure III.1 (9 months). Overall, girls seemed to be more sensitive to negative effects of prenatal OP exposure on 9-month motor function than boys (Figure III.1). For example, 9-month V-M scores were 1.69 points lower per 1 ng/mL increase in log-naled for girls (p=0.04) compared to only 0.06 points lower for boys (p=0.91). For girls,

estimated changes in V-M, FM, FMQ, and TMQ at 9 months were negative for all OPs examined. Chlorpyrifos consistently yielded deficits in 9-month motor scores, regardless of sex, though results were stronger in girls for many PDMS outcomes (stationary, locomotion, GM, GMQ, FMQ, TMQ). 9-month raw V-M, FM, and TM, results were statistically significant for both sexes. Scores were 1.96 (p=0.02) and 1.90 (p=0.009) points lower for V-M, 2.76 (p=0.03) and 2.66 (p=0.03) points lower for FM, and 8.16 (p=0.003) and 4.61 (p=0.06) points lower for TM, for chlorpyrifos-exposed girls and boys, respectively, compared to unexposed infants of the same sex.

Bivariate analyses showed no significant differences in INFANIB scores by infant sex (Table III.A7). Sex-stratified LMM results for OP/INFANIB associations are shown in Figure III.2 (6 weeks) and Table III.A8 (9 months). No clear sexually dimorphic trends were evident, except for 6-week total INFANIB score. For girls, estimated changes in total score were negative at 6 weeks for all OPs examined (-0.43, -0.41, -0.92, -1.79, -0.72, and -2.44 for total OPs, log-naled, methamidophos, trichlorfon, chlorpyrifos and phorate, respectively).

Odds of an abnormal PDMS-2 or INFANIB outcome are shown in Tables III.5 and III.6, respectively. Infants prenatally exposed to chlorpyrifos had 2.79 (95% CI: 1.16, 6.75) higher odds of an abnormally low GMQ at 9 months, compared to infants who were not exposed (Table III.5). Odds of an abnormally low INFANIB score were consistently higher with greater OP exposure, though not statistically significant (Table III.6).

Discussion

Prenatal naled and chlorpyrifos exposure was associated with decreased motor function in 9-month-old infants. For naled, negative effects were observed for fine motor outcomes, while chlorpyrifos was associated with deficits in both gross and fine motor function. Girls appeared to be more sensitive to the effects of prenatal OP exposure than boys. Additionally, odds of abnormal 9-month GMQ was nearly three times higher in infants prenatally exposed to chlorpyrifos, compared to those who were not exposed. No significant findings were observed at 6-weeks. While PDMS-2 is indicated for use in children from birth to five years, test validity and reliability to tend to be low in infants less than 6 months of age, possibly contributing to the lack of findings at the early time point (Folio & Fewell, 2000; Snider, Majnemer, Mazer, Campbell, & Bos, 2009).

No statistically significant associations were observed between prenatal OP exposure and infant INFANIB scores at either time point, though trends revealed that infants with more OP exposure had lower INFANIB scores and higher odds of an abnormal score at 6 weeks of age. Sex-specific findings for total INFANIB score were similar to those seen for PDMS-2.

Of the OPs measured in this study, 7 have been previously reported in cord blood in U.S. or Chinese cohorts. A comparison of the high ends of the exposure distributions across these studies has been published elsewhere (Silver, et al., 2016). For the current study, the 90th percentile concentration for chlorpyrifos (1.92 ng/mL) is several orders of magnitude higher than the

maximums reported in the U.S. (0.002-0.065 ng/mL) (Neta, et al., 2010; Whyatt, et al., 2003; Young, et al., 2005). Cord blood naled has not previously been reported.

Chlorpyrifos is employed widely in agricultural, land management, industrial, and vector control settings (NPIC, 2010). However, concerns of developmental neurotoxicity have led more governments to restrict its use over the past couple of decades (U.S. EPA, 2015). While chlorpyrifos has been highly studied as a neurotoxicant, relatively little has been published about naled. Naled's primary use is controlling adult mosquito populations (EPA, 2016). It has been employed for routine mosquito control in the U.S. and following hurricanes and floods (EPA, 2016) and is currently being sprayed aerially in southern Florida (U.S.) as part of a campaign to combat the spread of Zika virus (Frieden, Schuchat, & Petersen, 2016). Both chlorpyrifos and naled are able to cross the placenta(Abdel-Rahman, et al., 2002; EXTOXNET, 1993).

The findings from the current study are consistent with previous literature. Similar to a study that found deficits in infant fine motor function following maternal occupational exposure to unspecified OPs during pregnancy (Handal, et al., 2008), we observed deficits in raw FM at 9 months following prenatal exposure to all of the OPs analyzed in our study, with statistically significant results for naled and chlorpyrifos. Naled and chlorpyrifos also contributed to significant deficits in raw V-M, a fine motor subtest, as well as marginally significant deficits in FMQ. The only previous study to also use cord blood to assign exposure found associations between chlorpyrifos and psychomotor

development in 3-year-olds (V. A. Rauh, et al., 2006). We observed deficits in most 9-month PDMS measures following prenatal chlorpyrifos exposure, even with our relatively modest sample size. Two previous studies reported associations between maternal total urinary DEPs during pregnancy and infant/newborn reflexes (Engel, et al., 2007; Young, et al., 2005); 2 of the 3 DEPs used for the total DEP measurement (Engel, et al., 2007; Young, et al., 2005) are non-specific metabolites of chlorpyrifos (Bradman, et al., 2011). We similarly observed significant deficits in 9-month reflexes in infants with prenatal exposure to chlorpyrifos.

While nearly all studies of prenatal OP exposure and motor-related functions control for sex in their analyses, few report sex-specific results (Eskenazi, et al., 2007; V. A. Rauh, et al., 2006; Y. Zhang, et al., 2014). The effect of prenatal OP exposure on psychomotor development did not differ by sex in two studies (Eskenazi, et al., 2007; V. A. Rauh, et al., 2006). However, a study that found inverse associations between reflexes and maternal urinary total DAPs during pregnancy reported that associations were slightly stronger for girls (Y. Zhang, et al., 2014). Interestingly, when total DMPs and total DEPs were examined separately, the authors found that DMPs were significantly associated with deficits in reflexes in girls, while DEPs were significantly associated with deficits in reflexes in boys (Y. Zhang, et al., 2014). We observed stronger negative effects of prenatal OP exposure on 9-month motor function in girls for nearly all of the motor outcomes examined in this study.

The mechanism of acute toxicity elicited by high exposures to OPs is well understood. OPs inhibit acetylcholinesterase (AChE), the enzyme responsible for terminating the neurotransmitter acetylcholine's activity. Without functional AChE, acetylcholine builds up in the synapse, leading to hyperstimulation of the cholinergic receptors at neuronal and neuromuscular junctions (Abdollahi & Karami-Mohajeri, 2012; Eddleston, Buckley, Eyer, & Dawson, 2008; Kamanyire & Karalliedde, 2004). Cholinergic toxicity, as a result of acute chlorpyrifos poisoning, can result in motor dysfunction such as incoordination, loss of reflexes, muscle twitching, tremors, and paralysis (Kamanyire & Karalliedde, 2004).

However, low dose exposure levels, typical of those seen in non-occupational settings like this study, do not usually elicit cholinergic toxicity or acetylcholinesterase inhibition, yet effects on motor function are still observed. Low-dose prenatal or neonatal chlorpyrifos administered to laboratory rodents has been associated with deficits in motor-related outcomes. One study reported significant effects on neonatal motor patterns and delayed motor maturation in mice, following prenatal exposure to chlorpyrifos (De Felice, et al., 2015). Another similarly found motor abnormalities in developing rats following neonatal chlorpyrifos exposure (Dam, Seidler, & Slotkin, 2000). Interestingly, these findings were sex-specific. Deficits in coordination were observed almost immediately following chlorpyrifos administration in female rats only, while a delayed effect on locomotion was seen in male rats only. Both of these effects were largely independent of cholinesterase inhibition (Dam, et al., 2000).

Laboratory animal data concerning the neuromotor effects of naled are scarce. California EPA government reports note that naled at high exposure at levels can elicit gait alterations, tremors, reduced grasp, and hypoactivity in adult rats of both sexes (CalEPA, 2004), while an additional study reported sporadic tremors in high-dose female, but not male, rats (ACGIH, 2013). We were unable to find any published toxicology studies of gestational or neonatal naled exposure and motor-related outcomes in animal models.

The mechanisms by which OPs elicit these low-dose effects on motor function are unclear. An in-depth examination is beyond the scope of this discussion, but rodent models have yielded some plausible mechanisms that deserve mention. Briefly, low-dose prenatal chlorpyrifos has been found to have long-lasting effects on monoaminergic pathways of the brain (Aldridge, Seidler, & Slotkin, 2004). Specifically, it has been shown to perturb the development of serotonin (5HT) receptor circuits in the developing rat brain, leading to dysfunction of 5HT systems and behavioral abnormalities (Slotkin & Seidler, 2007b). These monoaminergic pathways play an important role in the maturation of spinal locomotor networks (De Felice, et al., 2015). Disruption of the timing of their development, as result of prenatal OP exposure, could have potentially negative consequences on early-life motor function. An alternative hypothesis is that OPs may affect motor function via the disruption of glial cell development and function in the brain (Garcia, Seidler, Crumpton, & Slotkin, 2001; Garcia, Seidler, Qiao, & Slotkin, 2002; Garcia, Seidler, & Slotkin, 2003; Zurich, Honegger, Schilter, Costa, & Monnet-Tschudi, 2004). Rodent studies

have shown that low-level chlopyrifos and diazinon exposure during the onset of myelination elicits deficits in expression of genes involved in oligodendrocyte function and myelination processes (Garcia, et al., 2001; Garcia, et al., 2002; Garcia, et al., 2003; Slotkin & Seidler, 2007a). Gains in motor function and mobility during infancy correspond to increases in corticospinal tract myelination, a process that begins in late pregnancy (Carlson, 2014; Dąmbska & Wisniewski, 1999). Therefore, prenatal OP exposure during the onset of corticospinal tract myelination has the potential to disrupt motor-related outcomes in infancy.

Our study is limited in several ways. The OPs measured here were non-persistent. Having measures of exposure at only a single time point (birth) limited our ability to address the temporal variability of OP exposure during pregnancy and infancy; thus, we may have missed some exposure at sensitive developmental stages (Eskenazi, et al., 2007). Since we measured a large number of pesticides with widely varying properties, our methods were not optimized solely for OP detection (Silver, et al., 2016). This likely resulted in higher detection limits and greater numbers of non-detects for some OPs, compared to a more targeted approach. In general, OP levels in blood tend to be low anyway, due to short half-lives (<48 hours), which also likely contributed to large number of non-detects (Barr, et al., 1999). This necessitated the use of crude exposure categories (<LOD/detect or <LOD/medium/high) for many of the OPs examined and limited the scope of our statistical analyses. We did not have information about parental interaction or play with the infants, while it may not directly confound the relationship between OP exposure and motor function, it is

almost certainly associated with the outcome and could have added precision to the estimates. Finally, the findings here may not be generalizable to infants born in other parts of China or elsewhere around the world, especially considering that all the infants included in this study were carried to term and otherwise healthy. Low birth weight or pre-term infants are more likely to have delayed or impaired motor development (Snider, et al., 2009) and may potentially be more susceptible to effects of prenatal pesticide exposures. The effects of prenatal OPs on infant motor function should be assessed in these vulnerable populations.

Despite its limitations, this study has a number of strengths. It used specific measurements of OP parent compounds in umbilical cord blood to assign prenatal exposure, rather than non-specific metabolites in maternal urine, thus providing direct evidence of fetal exposure (Barr, et al., 1999; Munoz-Quezada, et al., 2013). OP levels in cord blood may also be more likely to reflect the available dose, since the measured OPs have not yet been eliminated from the infant's body (Needham, Ashley, & Patterson, 1995). Of the previously published studies of prenatal OPs and motor function, only one directly measured concentrations of the parent pesticide in cord blood to assign prenatal exposure (V. A. Rauh, et al., 2006); other studies used non-specific urinary metabolites during pregnancy (Engel, et al., 2007; Engel, et al., 2011; Eskenazi, et al., 2010; Eskenazi, et al., 2007; Young, et al., 2005; Y. Zhang, et al., 2014) or maternal occupation (Handal, et al., 2008; Harari, et al., 2010) to assign exposure. This study also examined a large number of OPs (18 detected out of

30 analyzed), many of which have not been previously investigated for neurodevelopmental effects in infants. To our knowledge, this is the first non-occupational human study of the health effects of naled. Additionally, we assessed motor development at two time points (6 weeks and 9 months). The longitudinal design, as well as the use of a motor test that was sensitive to changes in both gross and fine motor function, gives a more comprehensive view of overall motor function in infancy than previous studies.

Conclusions

Prenatal naled and chlorpyrifos exposure was significantly associated with decreased motor function in Chinese infants. The clinical significance of these small, yet significant, deficits in infant motor development are unknown. Both chlorpyrifos and naled are used around the world. These results warrant further exploration of the effects of commonly used OPs on motor development. Proper motor skill acquisition in infancy is essential to downstream neurodevelopment and cognitive processes.

Acknowledgments

This study was funded by R01ES021465 from the National Institute of Environmental Health Sciences (NIEHS), P01HD39386 from the National

Institute of Child Health and Development (NICHD), and 81273085 from the National Natural Science Foundation of China (NNSFC).

I would also like to acknowledge my collaborators and coauthors on this work: Jie Shao, Binquan Zhu, Minjian Chen, Yankai Xia, Niko Kaciroti, Betsy Lozoff and John Meeker. I would additionally like to thank Rosa-Angulo Barroso for her assistance in interpreting the tests of motor function used here.

References

Abdel-Rahman, A. A., Blumenthal, G. M., Abou-Donia, S. A., Ali, F. A., Abdel-Monem, A. E., & Abou-Donia, M. B. (2002). Pharmacokinetic profile and placental transfer of a single intravenous injection of [(14)C]chlorpyrifos in pregnant rats. *Arch Toxicol, 76*(8), 452-459.

Abdollahi, M., & Karami-Mohajeri, S. (2012). A comprehensive review on experimental and clinical findings in intermediate syndrome caused by organophosphate poisoning. *Toxicol Appl Pharmacol, 258*(3), 309-314.

ACGIH. (2013). *Documentation of the TLVs and BEIs with other world wide occupational exposure values*. Cincinnati, Ohio: American Conference of Governmental Industrial Hygienists.

Aldridge, J. E., Seidler, F. J., & Slotkin, T. A. (2004). Developmental exposure to chlorpyrifos elicits sex-selective alterations of serotonergic synaptic function in adulthood: critical periods and regional selectivity for effects on the serotonin transporter, receptor subtypes, and cell signaling. *Environ Health Perspect, 112*(2), 148-155.

Bai, L., Morton, L. C., & Liu, Q. (2013). Climate change and mosquito-borne diseases in China: a review. *Global Health, 9*, 10.

Barr, D. B., Barr, J. R., Driskell, W. J., Hill, R. H., Jr., Ashley, D. L., Needham, L. L., et al. (1999). Strategies for biological monitoring of exposure for contemporary-use pesticides. *Toxicol Ind Health, 15*(1-2), 168-179.

Bouchard, M. F., Chevrier, J., Harley, K. G., Kogut, K., Vedar, M., Calderon, N., et al. (2011). Prenatal exposure to organophosphate pesticides and IQ in 7-year-old children. *Environ Health Perspect, 119*(8), 1189-1195.

Bradman, A., Castorina, R., Barr, D. B., Chevrier, J., Harnly, M. E., Eisen, E. A., et al. (2011). Determinants of organophosphorus pesticide urinary metabolite levels in young children living in an agricultural community. *Int J Environ Res Public Health, 8*(4), 1061-1083.

CalEPA. (2004). *Summary of Toxicology Data for Naled, Chemical Code No.418*. Retrieved from http://www.cdpr.ca.gov/docs/risk/toxsums/pdfs/418.pdf.

Carlson, B. M. (2014). *Human Embryology and Developmental Biology* (5th ed.): Elsevier.

Clearfield, M. W. (2004). The role of crawling and walking experience in infant spatial memory. *J Exp Child Psychol, 89*(3), 214-241.

Clearfield, M. W. (2011). Learning to walk changes infants' social interactions. *Infant Behav Dev, 34*(1), 15-25.

Dam, K., Seidler, F. J., & Slotkin, T. A. (2000). Chlorpyrifos exposure during a critical neonatal period elicits gender-selective deficits in the development of coordination skills and locomotor activity. *Brain Res Dev Brain Res, 121*(2), 179-187.

Dąmbska, M., & Wisniewski, K. E. (1999). *Normal and Pathologic Development of the Human Brain and Spinal Cord*. London, England: John LIbby & Co. Ltd.

De Felice, A., Scattoni, M. L., Ricceri, L., & Calamandrei, G. (2015). Prenatal exposure to a common organophosphate insecticide delays motor development in a mouse model of idiopathic autism. *PLoS One, 10*(3), e0121663.

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

Eddleston, M., Buckley, N. A., Eyer, P., & Dawson, A. H. (2008). Management of acute organophosphorus pesticide poisoning. *Lancet, 371*(9612), 597-607.

Ellison, P. H. (1986). Scoring sheet for the Infant Neurological International Battery (INFANIB). Suggestion from the field. *Phys Ther, 66*(4), 548-550.

Ellison, P. H., Horn, J. L., & Browning, C. A. (1985). Construction of an Infant Neurological International Battery (Infanib) for the assessment of neurological integrity in infancy. *Phys Ther, 65*(9), 1326-1331.

Engel, S. M., Berkowitz, G. S., Barr, D. B., Teitelbaum, S. L., Siskind, J., Meisel, S. J., et al. (2007). Prenatal organophosphate metabolite and organochlorine levels and performance on the Brazelton Neonatal Behavioral Assessment Scale in a multiethnic pregnancy cohort. *Am J Epidemiol, 165*(12), 1397-1404.

Engel, S. M., Wetmur, J., Chen, J., Zhu, C., Barr, D. B., Canfield, R. L., et al. (2011). Prenatal exposure to organophosphates, paraoxonase 1, and cognitive development in childhood. *Environ Health Perspect, 119*(8), 1182-1188.

Eskenazi, B., Huen, K., Marks, A., Harley, K. G., Bradman, A., Barr, D. B., et al. (2010). PON1 and neurodevelopment in children from the CHAMACOS study exposed to organophosphate pesticides in utero. *Environ Health Perspect, 118*(12), 1775-1781.

Eskenazi, B., Marks, A. R., Bradman, A., Harley, K., Barr, D. B., Johnson, C., et al. (2007). Organophosphate pesticide exposure and neurodevelopment in young Mexican-American children. *Environ Health Perspect, 115*(5), 792-798.

EXTOXNET. (1993). Naled pesticide information profile. Retrieved October 31, 2016, from www.pmep.cce.cornell.edu/profiles/extoxnet/metiram-propoxur/naled-ext.html

Folio, M. R., & Fewell, R. R. (1983). *Peabody Developmental Motor Scales and Actvity Cards*. Chicago, IL: Riverside.

Folio, M. R., & Fewell, R. R. (2000). *Peabody Developmental Motor Scales Examiner's Manual 2nd ed.* Austin, TX: Pro-Ed.

Frieden, T. R., Schuchat, A., & Petersen, L. R. (2016). Zika Virus 6 Months Later. *JAMA, 316*(14), 1443-1444.

Garcia, S. J., Seidler, F. J., Crumpton, T. L., & Slotkin, T. A. (2001). Does the developmental neurotoxicity of chlorpyrifos involve glial targets? Macromolecule synthesis, adenylyl cyclase signaling, nuclear transcription factors, and formation of reactive oxygen in C6 glioma cells. *Brain Res, 891*(1-2), 54-68.

Garcia, S. J., Seidler, F. J., Qiao, D., & Slotkin, T. A. (2002). Chlorpyrifos targets developing glia: effects on glial fibrillary acidic protein. *Brain Res Dev Brain Res, 133*(2), 151-161.

Garcia, S. J., Seidler, F. J., & Slotkin, T. A. (2003). Developmental neurotoxicity elicited by prenatal or postnatal chlorpyrifos exposure: effects on neurospecific proteins indicate changing vulnerabilities. *Environ Health Perspect, 111*(3), 297-303.

Garcia, S. J., Seidler, F. J., & Slotkin, T. A. (2005). Developmental neurotoxicity of chlorpyrifos: targeting glial cells. *Environ Toxicol Pharmacol, 19*(3), 455-461.

Handal, A. J., Harlow, S. D., Breilh, J., & Lozoff, B. (2008). Occupational exposure to pesticides during pregnancy and neurobehavioral development of infants and toddlers. *Epidemiology, 19*(6), 851-859.

Harari, R., Julvez, J., Murata, K., Barr, D., Bellinger, D. C., Debes, F., et al. (2010). Neurobehavioral deficits and increased blood pressure in school-age children prenatally exposed to pesticides. *Environ Health Perspect, 118*(6), 890-896.

Huang, J., Qiao, F., Zhang, L., & Rozelle, S. (2001). *Farm pesticide, rice production, and human health*.

Kamanyire, R., & Karalliedde, L. (2004). Organophosphate toxicity and occupational exposure. *Occup Med (Lond), 54*(2), 69-75.

Marks, A. R., Harley, K., Bradman, A., Kogut, K., Barr, D. B., Johnson, C., et al. (2010). Organophosphate pesticide exposure and attention in young Mexican-American children: the CHAMACOS study. *Environ Health Perspect, 118*(12), 1768-1774.

Munoz-Quezada, M. T., Lucero, B. A., Barr, D. B., Steenland, K., Levy, K., Ryan, P. B., et al. (2013). Neurodevelopmental effects in children associated with exposure to organophosphate pesticides: a systematic review. *Neurotoxicology, 39*, 158-168.

Needham, L. L., Ashley, D. L., & Patterson, D. G., Jr. (1995). Case studies of the use of biomarkers to assess exposures. *Toxicol Lett, 82-83*, 373-378.

Neta, G., Goldman, L. R., Barr, D., Sjodin, A., Apelberg, B. J., Witter, F. R., et al. (2010). Distribution and determinants of pesticide mixtures in cord serum using principal component analysis. *Environ Sci Technol, 44*(14), 5641-5648.

Noritz, G. H., & Murphy, N. A. (2013). Motor delays: early identification and evaluation. *Pediatrics, 131*(6), e2016-2027.

NPIC. (2010). Chlorpyrifos General Fact Sheet. Retrieved June 9, 2016, from www.npic.orst.edu/factsheets/chlorpgen.html

Perez, J. J., Williams, M. K., Weerasekera, G., Smith, K., Whyatt, R. M., Needham, L. L., et al. (2010). Measurement of pyrethroid, organophosphorus, and carbamate insecticides in human plasma using isotope dilution gas chromatography-high resolution mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci, 878*(27), 2554-2562.

Rauh, V., Arunajadai, S., Horton, M., Perera, F., Hoepner, L., Barr, D. B., et al. (2011). Seven-year neurodevelopmental scores and prenatal exposure to

chlorpyrifos, a common agricultural pesticide. *Environ Health Perspect, 119*(8), 1196-1201.

Rauh, V. A., Garfinkel, R., Perera, F. P., Andrews, H. F., Hoepner, L., Barr, D. B., et al. (2006). Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. *Pediatrics, 118*(6), e1845-1859.

Shelton, J. F., Geraghty, E. M., Tancredi, D. J., Delwiche, L. D., Schmidt, R. J., Ritz, B., et al. (2014). Neurodevelopmental disorders and prenatal residential proximity to agricultural pesticides: the CHARGE study. *Environ Health Perspect, 122*(10), 1103-1109.

Silver, M. K., Shao, J., Chen, M., Xia, Y., Lozoff, B., & Meeker, J. D. (2016). Distribution and Predictors of Pesticides in the Umbilical Cord Blood of Chinese Newborns. *Int J Environ Res Public Health, 13*(1).

Slotkin, T. A., & Seidler, F. J. (2007a). Comparative developmental neurotoxicity of organophosphates in vivo: transcriptional responses of pathways for brain cell development, cell signaling, cytotoxicity and neurotransmitter systems. *Brain Res Bull, 72*(4-6), 232-274.

Slotkin, T. A., & Seidler, F. J. (2007b). Prenatal chlorpyrifos exposure elicits presynaptic serotonergic and dopaminergic hyperactivity at adolescence: critical periods for regional and sex-selective effects. *Reprod Toxicol, 23*(3), 421-427.

Snider, L., Majnemer, A., Mazer, B., Campbell, S., & Bos, A. F. (2009). Prediction of motor and functional outcomes in infants born preterm assessed at term. *Pediatr Phys Ther, 21*(1), 2-11.

ThermoScientific. Rapid analysis of pesticides in difficult matrices using GC/MS/MS. Retrieved September 15, 2013, from http://www.analiticaweb.com.br/newsletter/09/51880_RapidAnalysisPesticides.pdf

U.S. CDC. (2016). Parasites- Lice- Head Lice- Treatment. Retrieved October 31, 2016, from www.cdc.gov/parasites/lice/head/treatment.html

U.S. EPA. (2011). Pesticide industry sales and usage: 2006 and 2007 market estimates. Retrieved March 13, 2013, from http://www.panna.org/sites/default/files/EPA%20market_estimates2007.pdf

U.S. EPA. (2015). EPA Revised Chlorpyrifos Assessment Shows Risk to Workers. Retrieved June 9, 2016, from https://yosemite.epa.gov/opa/admpress.nsf/d0cf6618525a9efb85257359003fb69d/6b1d8a363e75a61e85257dc4006b1524!OpenDocument

U.S. EPA. (2016). Naled for Mosquito Control. Retrieved October 10, 2016, from https://www.epa.gov/mosquitocontrol/naled-mosquito-control

Whyatt, R. M., Barr, D. B., Camann, D. E., Kinney, P. L., Barr, J. R., Andrews, H. F., et al. (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*(5), 749-756.

Wickerham, E. L., Lozoff, B., Shao, J., Kaciroti, N., Xia, Y., & Meeker, J. D. (2012). Reduced birth weight in relation to pesticide mixtures detected in cord blood of full-term infants. *Environ Int, 47*, 80-85.

Young, J. G., Eskenazi, B., Gladstone, E. A., Bradman, A., Pedersen, L., Johnson, C., et al. (2005). Association between in utero organophosphate pesticide exposure and abnormal reflexes in neonates. *Neurotoxicology, 26*(2), 199-209.

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*(2), 125-144.

Zhang, Y., Han, S., Liang, D., Shi, X., Wang, F., Liu, W., et al. (2014). Prenatal exposure to organophosphate pesticides and neurobehavioral development of neonates: a birth cohort study in shenyang, china. *PLoS One, 9*(2), e88491.

Zurich, M. G., Honegger, P., Schilter, B., Costa, L. G., & Monnet-Tschudi, F. (2004). Involvement of glial cells in the neurotoxicity of parathion and chlorpyrifos. *Toxicol Appl Pharmacol, 201*(2), 97-104.

Table III.1. Study sample characteristics

Demographics			Exposures (cord blood)		
Variable	**N**	**Mean (SD)**	**Variable**	**N**	**Mean (SD)**
Age (days) at 6 week testing	204	43.1 (5.4)	# OP detects	206	2.9 (1.6)
Age (days) at 9 month testing	205	283.0 (10.6)	Naled (ng/mL)	200	2.1 (2.7) [GM]
Gestational age (weeks)	206	39.6 (1.0)			**N (%)**
Birth weight (kg)	233	3.4 (0.4)	Methamidophos (ng/mL)	206	
			High (>18.2)		66 (32.0)
		N (%)	Medium (1.5-18.2)		66 (32.0)
Sex	206		<LOD (<1.5)		74 (35.9)
Male		106 (51.5)	Trichlorfon (ng/mL)	206	
Female		100 (48.5)	High (>1.7)		49 (23.8)
Maternal occupation	194		Medium (0.4-1.7)		55 (26.7)
Housewife		84 (43.3)	<LOD (<0.4)		74 (35.9)
Other		110 (56.7)	Chlorpyrifos (ng/mL)	206	
Maternal education	194		Detect (≥0.4)		71 (34.5)
College		63 (32.5)	ND (<0.4)		135 (65.5)
High/secondary school		55 (28.4)	Phorate (ng/mL)	206	
Middle school or less		76 (39.2)	Detect (≥1.8)		32 (15.5)
Family income (Yuan/year)	189		ND (<1.8)		174 (84.5)
≥ 100,000		57 (30.2)	Serum ferritin (μg/L)	193	
50,000-999,999		55 (29.1)	Normal (75-370)		157 (81.3)
30,000-49,999		37 (19.6)	Low (≤75)		36 (18.7)
<30,000		40 (20.2)			

[GM] Denotes geometric mean

Table III.2. Distribution of OP insecticide concentrations in umbilical cord blood plasma samples (ng/mL) at delivery, Zhejiang Province, China (n=237)

Organophosphate	LOD	N >LOD (%)	50th	75th	90th	95th	99th	Max
Acephate	0.10	7 (3.0)	ND	ND	ND	ND	0.57	0.68
Chlorpyrifos	0.40	87 (36.7)	ND	0.56	1.92	2.71	4.65	7.33
Chlorpyrifos-methyl	0.01	18 (7.6)	ND	ND	ND	0.07	0.47	1.14
Fensulfothion	0.03	1 (0.4)	ND	ND	ND	ND	ND	10.35
Fosthiazate	0.07	1 (0.4)	ND	ND	ND	ND	ND	7.82
Isofenphos-methyl	0.13	2 (0.8)	ND	ND	ND	ND	ND	14.70
Methamidophos	1.52	153 (64.6)	6.11	28.10	59.60	113.71	231.96	496.86
Methidathion	0.07	1 (0.4)	ND	ND	ND	ND	ND	9.23
Mevinphos	0.12	15 (6.3)	ND	ND	ND	0.14	0.25	0.26
Naled	0.42	236 (99.6)	1.51	4.33	9.17	12.22	22.13	29.23
Omethoate	1.35	19 (8.0)	ND	ND	ND	1.83	4.37	9.34
Phorate	1.79	40 (16.9)	ND	ND	3.10	5.14	7.02	12.82
Terbufos	0.33	3 (1.3)	ND	ND	ND	ND	0.39	3.32
Trichlorfon	0.35	121 (51.0)	0.46	1.69	3.65	5.52	10.56	11.30
*Carbophenothion sulfone	0.02	16 (6.8)	ND	ND	ND	0.49	1.64	18.83
*DEDTP	0.06	2 (0.8)	ND	ND	ND	ND	ND	0.69
*DMTP	1.35	1 (0.4)	ND	ND	ND	ND	ND	9.24
*TCPY	2.32	1 (0.4)	ND	ND	ND	ND	ND	13.52
Undetected: Diazinon, Dicrotophos, Dimethoate, Formothion, Phosphamidon, Dimethylvinphos, Parathion-methyl, Malathion, Dichlorphos, Monocrotophos, *Phorate sulfone, *DMDTP								

* Denotes a metabolite
ND, non-detect; DEDTP, diethyldithiophosphate; DMTP, dimethylthiophosphate; TCPY, 3,5,6-trichloro-2-pyridinol; DMDTP, dimethyldithiophosphate

Table III.3. Adjusted [a] change/difference in PDMS motor scores at 9 months by OP exposure

	Raw Subtest Scores				
OP Insecticide	**Reflexes (n=182)**	**Stationary (n=188)**	**Locomotion (n=187)**	**Grasping (n=191)**	**V-M (n=192)**
Continuous	**β (95% CI) for OP [b]**				
# OP Detects	0.03	0.08	0.04	-0.08	-0.21
	(-0.10, 0.17)	(-0.18, 0.33)	(-0.44-0.53)	(-0.32, 0.17)	(-0.54, 0.13)
Log-Naled	0.08	0.07	0.06	-0.30	-0.55 *
	(-0.14, 0.30)	(-0.33, 0.47)	(-0.71, 0.83)	(-0.68, 0.08)	(-1.07, -0.30)
3-level (High/Med./ND)	**Difference in least square means (95% CI) [c]**				
Methamidophos	-0.12	-0.31	-1.03	-0.70	-0.55
(High vs ND)	(-0.66, 0.41)	(-1.29, 0.67)	(-2.91, 0.85)	(-1.63, 0.23)	(-1.83, 0.73)
Methamidophos	0.06	-0.13	0.13	-0.13	-0.01
(Med. vs ND)	(-0.48, 0.59)	(-1.10, 0.84)	(-1.73, 1.99)	(-1.06, 0.79)	(-1.28, 1.26)
Trichlorfon	0.28	0.59	1.19	0.08	-0.10
(High vs ND)	(-0.27, 0.83)	(-0.40, 1.58)	(-0.71, 3.09)	(-0.87, 1.03)	(-1.40, 1.21)
Trichlorfon	0.30	-0.17	-0.06	-0.54	0.07
(Med. vs ND)	(-0.23, 0.84)	(-1.14, 0.80)	(-1.92, 1.81)	(-1.46, 0.39)	(-1.34, 1.21)
2-level (Detect/ND)	**Difference in least square means (95% CI) [c]**				
Chlorpyrifos	-0.50 *	-0.67	-1.98 *	-0.80 †	-1.91 ***
(Detect vs ND)	(-0.96, 0.04)	(-1.52, 0.18)	(-3.62, -0.35)	(-1.61, 0.01)	(-3.01, -0.81)
Phorate	0.22	0.70	0.49	0.66	-1.01
(Detect vs ND)	(-0.39, 0.82)	(-0.40, 1.80)	(-1.64, 2.61)	(-0.40, 1.73)	(-2.45, 0.44)

[a] Models adjusted for sex, age at testing, and cord ferritin
[b] Estimated change in 9-month motor score per 1 unit increase in OP
[c] Difference in mean 9-month motor score
High/Medium/ND cut-offs (ng/mL): methamidophos >18.2/1.5-18.2/ND; trichlorfon >1.7/ 0.4-1.7/ND; chlorpyrifos ≥0.04/ND; 7phorate ≥1.8/ND
Abbreviations: V-M, visual-motor integration
†p<0.10, *p<0.05, **p<0.01, ***p<0.001

Table III.3, continued

OP Insecticide	Composite Raw Scores			Motor Quotients		
	GM (n=180)	FM (n=191)	TM (n=179)	GMQ (n=180)	FMQ (n=191)	TMQ (n=179)
Continuous	**β (95% CI) for OP [b]**					
# OP Detects	0.12	-0.29	-0.14	0.11	-0.23	-0.03
	(-0.64, 0.88)	(-0.80, 0.23)	(-1.21, 0.93)	(-0.48, 0.69)	(-0.89, 0.43)	(-0.60, 0.53)
Log-Naled	0.36	-0.85 *	-0.53	0.44	-0.90 †	-0.10
	(-0.84, 1.55)	(-1.65, -0.06)	(-2.20, 1.15)	(-0.48, 1.36)	(-1.92, 0.12)	(-0.99, 0.79)
3-level (High/Med./ND)	**Difference in least square means (95% CI) [c]**					
Methamidophos	-1.30	-1.25	-2.77	-1.32	-1.67	-0.62
(High vs ND)	(-4.24, 1.65)	(-3.21, 0.71)	(-6.89, 1.36)	(-3.51, 0.87)	(-4.18, 0.85)	(-2.90, 1.67)
Methamidophos	0.41	-0.18	0.46	0.07	0.25	-0.23
(Med. vs ND)	(-2.53, 3.35)	(-2.11, 1.77)	(-3.64, 4.57)	(-2.12, 2.25)	(-2.25, 2.75)	(-2.52, 2.06)
Trichlorfon	2.12	-0.05	2.39	2.07	0.70	1.77
(High vs ND)	(-0.88, 5.12)	(-2.06, 1.96)	(-1.84, 6.61)	(-0.24, 4.38)	(-1.87, 3.26)	(-0.46, 4.00)
Trichlorfon	-0.28	-0.60	-0.75	-0.26	-0.97	-0.50
(Med. vs ND)	(3.20, 2.64)	(-2.55, 1.35)	(-4.84, 3.33)	(-2.53, 2.01)	(-3.47, 1.54)	(-2.67-1.67)
2-level (Detect/ND)	**Difference in least square means (95% CI) [c]**					
Chlorpyrifos	-3.49 **	-2.71 **	-6.29 ***	-2.56 *	-2.04 †	-2.59 **
(Detect vs ND)	(-6.03, -0.95)	(-4.40, -1.02)	(-9.83, -2.75)	(-4.53, -0.59)	(-4.23, 0.15)	(-4.49, -0.70)
Phorate	1.28	-0.38	1.07	0.79	0.51	0.69
(Detect vs ND)	(-2.00, 4.57)	(-2.62, 1.86)	(-3.59, 5.73)	(-1.74, 3.33)	(-2.35, 3.37)	(-1.77, 3.16)

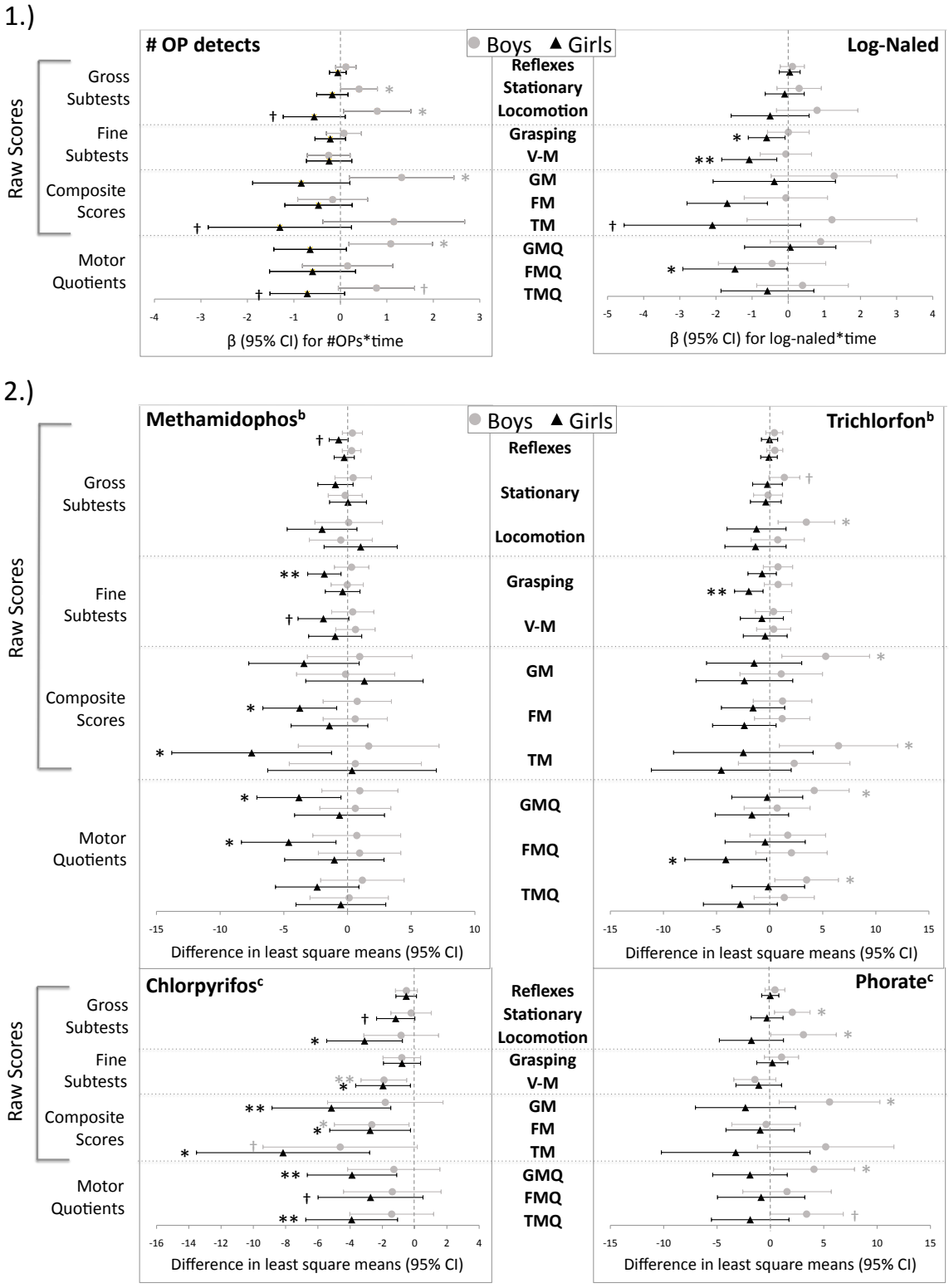
[a] Models adjusted for sex, age at testing, and cord ferritin
[b] Estimated change in 9-month motor score per 1 unit increase in OP
[c] Difference in mean 9-month motor score
High/Medium/ND cut-offs (ng/mL): methamidophos >18.2/1.5-18.2/ND; trichlorfon >1.7/ 0.4-1.7/ND; chlorpyrifos ≥0.04/ND; phorate ≥1.8/ND
Abbreviations: GM, gross motor score; FM, fine motor score; TM, total motor score; GMQ, gross motor quotient;
FMQ, fine motor quotient, TMQ, total motor quotient
†p<0.10, *p<0.05, **p<0.01, ***p<0.001

Table III.4. Adjusted [a] change/difference in INFANIB scores at 6 weeks by OP exposure

	Subscale scores				Total score
OP insecticide	**Spasticity (n=197)**	**Head/trunk (n=197)**	**Legs (n=197)**	**French angles (n=197)**	**Total (n=197)**
Continuous	**β (95% CI) for OP [b]**				
# OP Detects	-0.01	-0.06	-0.04	-0.13	-0.24
	(-0.10, 0.08)	(-0.23, 0.12)	(-0.20, 0.11)	(-0.38, 0.13)	(-0.82, 0.33)
Log-Naled	-0.08	0.08	-0.13	-0.27	-0.44
	(-0.22, 0.07)	(-0.20, 0.35)	(-0.37, 0.12)	(-0.68, 0.14)	(-1.34, 0.47)
3-level (High/Med./ND)	**Difference in least square means (95% CI) [c]**				
Methamidophos	-0.16	-0.05	-0.01	-0.28	-0.48
(High vs ND)	(-0.52, 0.19)	(-0.74, 0.63)	(-0.61, 0.60)	(-1.30, 0.74)	(-2.74, 1.78)
Methamidophos	0.23	-0.11	-0.03	0.10	0.10
(Med. vs ND)	(-0.13, 0.58)	(-0.79, 0.56)	(-0.63, 0.58)	(-0.91, 1.11)	(-2.14, 2.34)
Trichlorfon	-0.04	-0.42	-0.17	-0.30	-1.15
(High vs ND)	(-0.40, 0.32)	(-1.10, 0.26)	(-0.78, 0.44)	(-1.32, 0.72)	(-3.42, 1.13)
Trichlorfon	0.10	0.04	0.28	-1.00 †	-0.59
(Med. vs ND)	(-0.25, 0.46)	(-0.64, 0.72)	(-0.33, 0.88)	(-2.02, 0.01)	(-2.85, 1.68)
2-level (Detect/ND)	**Difference in least square means (95% CI) [c]**				
Chlorpyrifos	0.01	-0.08	-0.12	-0.32	-0.47
(Detect vs ND)	(-0.29, 0.32)	(-0.66, 0.51)	(-0.64, 0.41)	(-1.19, 0.55)	(-2.41, 1.47)
Phorate	-0.21	-0.38	-0.48	-0.49	-1.52
(Detect vs ND)	(-0.59, 0.17)	(-1.11, 0.34)	(-1.13, 0.16)	(-1.56, 0.59)	(-3.91, 0.87)

[a] Models adjusted for sex, age at testing, and cord ferritin
[b] Estimated change in 6-week INFANIB score per 1 unit increase in OP
[c] Difference in mean 6-week INFANIB score
High/Medium/ND cut-offs (ng/mL): methamidophos >18.2/1.5-18.2/ND; trichlorfon >1.7/ 0.4-1.7/ND; chlorpyrifos ≥0.04/ND; phorate ≥1.8/ND
†p<0.10

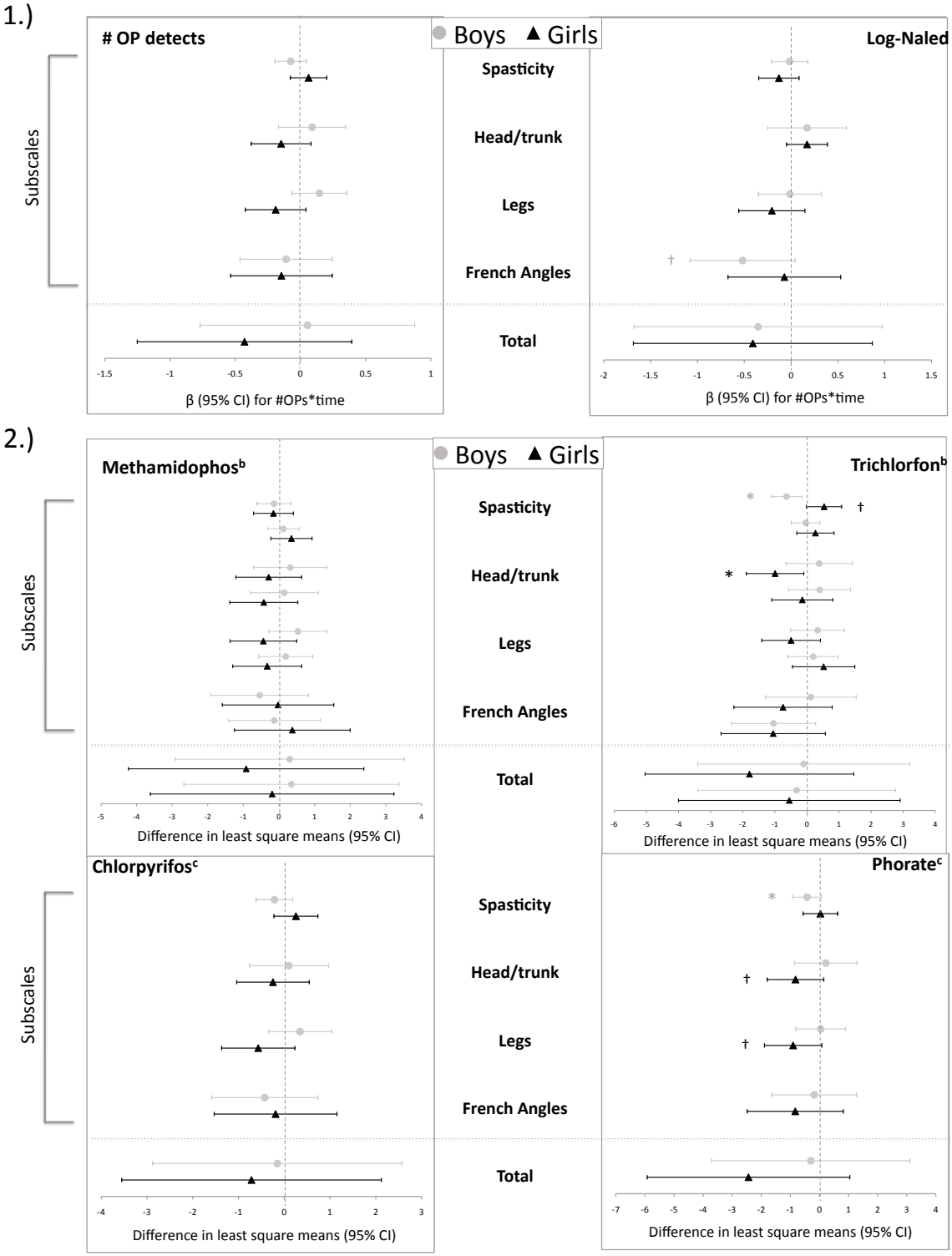
Figure III.1. Sex-stratified change/difference (95%) in PDMS motor scores at 9 months by OP exposure [a]



1.) Estimated change in 9-month PDMS motor score per 1 unit increase in OP exposure
2.) Difference in mean 9-month PDMS motor score by category of OP exposure

[a] Models adjusted for age at testing and cord ferritin
[b] Categories of OP exposure: high versus ND and medium versus ND
[c] Categories of OP exposure: exposed versus ND
High/Medium/ND cut-offs (ng/mL): methamidophos >18.2/1.5-18.2/ND; trichlorfon >1.7/ 0.4-1.7/ND; chlorpyrifos ≥0.04/ND; phorate ≥1.8/ND
†p<0.10, *p<0.05, **p<0.01

Figure III.2. Sex-stratified change/difference (95%) in INFANIB motor scores at 6 weeks by OP exposure [a]



1.) Estimated change in 6-week INFANIB motor score per 1 unit increase in OP exposure
2.) Difference in mean 9-month INFANIB motor score by category of OP exposure

[a] Models adjusted for age at testing and cord ferritin
[b] Categories of OP exposure: high versus ND and medium versus ND
[c] Categories of OP exposure: exposed versus ND
High/Medium/ND cut-offs (ng/mL): methamidophos >18.2/1.5-18.2/ND; trichlorfon >1.7/ 0.4-1.7/ND; chlorpyrifos ≥0.04/ND; phorate ≥1.8/ND
†p<0.10, *p<0.05

Table III.5. Adjusted [a] odds (95% CI) of clinically defined abnormally low PDMS scores at 6-weeks or 9-months by OP exposure

	6 weeks				
	Standardized subtest scores			Motor quotients	
OP insecticide	**Stationary (n=197)**	**Grasping (n=197)**	**V-M (n=197)**	**FMQ (n=197)**	**TMQ (n=197)**
Continuous	**OR (95% CI)**				
# OP Detects	1.01 (0.81, 1.22)	0.98 (0.82, 1.16)	1.04 (0.87, 1.25)	0.98 (0.82, 1.17)	1.02 (0.85, 1.24)
Log-Naled	1.22 (0.80, 1.70)	0.96 (0.73, 1.26)	0.84 (0.62, 1.14)	0.79 (0.59, 1.05)	0.93 (0.69, 1.26)
3-level (High/Med./ND)	**OR (95% CI)**				
Methamidophos (High vs ND)	1.05 (0.48, 2.31)	0.63 (0.32, 1.27)	0.94 (0.44, 1.99)	0.82 (0.41, 1.66)	1.15 (0.55, 2.42)
Methamidophos (Med. vs ND)	1.47 (0.65, 3.31)	0.79 (0.40, 1.58)	1.37 (0.67, 2.80)	0.95 (0.48, 1.90)	1.01 (0.48, 2.13)
Trichlorfon (High vs ND)	0.77 (0.35, 1.70)	1.08 (0.53, 2.19)	0.82 (0.38, 1.79)	1.46 (0.72, 2.97)	1.29 (0.61, 2.72)
Trichlorfon (Med. vs ND)	1.21 (0.52, 2.82)	1.01 (0.50, 2.02)	1.63 (0.80, 3.34)	1.41 (0.70, 2.82)	1.06 (0.50, 2.25)
2-level (Detect/ND)	**OR (95% CI)**				
Chlorpyrifos (Detect vs ND)	0.98 (0.49, 1.96)	1.17 (0.65, 2.13)	0.80 (0.42, 1.52)	0.82 (0.45, 1.50)	1.18 (0.62, 2.22)
Phorate (Detect vs ND)	0.86 (0.37, 2.00)	0.71 (0.34, 1.49)	0.99 (0.45, 2.16)	0.65 (0.30, 1.40)	0.72 (0.31, 1.66)

[a] Models adjusted for sex, age at testing, and cord ferritin
High/Medium/ND cut-offs (ng/mL): methamidophos >18.2/1.5-18.2/ND; trichlorfon >1.7/ 0.4-1.7/ND; chlorpyrifos ≥0.04/ND; phorate ≥1.8/ND

Table III.5, continued

	9 months		
	Standardized subtest scores		Motor quotient
OP insecticide	**Reflexes (n=182)**	**Locomotion (n=187)**	**GMQ (n=180)**
Continuous	**OR (95% CI)**		
# OP Detects	0.99 (0.74, 1.33)	0.86 (0.72, 1.04)	1.02 (0.78, 1.33)
Log-Naled	0.93 (0.58, 1.50)	0.85 (0.64, 1.15)	0.96 (0.62, 1.48)
3-level (High/Med./ND)	**OR (95% CI)**		
Methamidophos (High vs ND)	1.02 (0.32, 3.29)	0.92 (0.45, 1.88)	0.89 (0.31, 2.59)
Methamidophos (Med. vs ND)	1.34 (0.45, 3.99)	0.96 (0.47, 1.95)	1.14 (0.42, 3.13)
Trichlorfon (High vs ND)	0.99 (0.32, 3.10)	0.66 (0.32, 1.35)	0.67 (0.22, 2.04)
Trichlorfon (Med. vs ND)	0.88 (0.28, 2.74)	0.72 (0.35, 1.47)	0.75 (0.26, 2.12)
2-level (Detect/ND)	**OR (95% CI)**		
Chlorpyrifos (Detect vs ND)	2.07 (0.81, 5.30)	0.91 (0.48, 1.68)	2.79 * (1.16, 6.75)
Phorate (Detect vs ND)	0.56 (0.12, 2.58)	0.96 (0.40, 2.01)	1.38 (0.46, 4.10)

[a] Models adjusted for sex, age at testing, and cord ferritin
High/Medium/ND cut-offs (ng/mL): methamidophos >18.2/1.5-18.2/ND; trichlorfon >1.7/ 0.4-1.7/ND; chlorpyrifos ≥0.04/ND; phorate ≥1.8/ND
*p<0.05

Table III.6. Adjusted [a] odds (95% CI) of clinically defined abnormally low INFANIB total score at 6-weeks or 9-months by OP exposure

OP insecticide	6 weeks (n=197)	9 months (n=184)
Continuous	**OR (95% CI)**	
# OP Detects	1.10 (0.89, 1.35)	0.84 (0.68, 1.05)
Log-Naled	1.19 (0.86, 1.65)	1.07 (0.78, 1.48)
3-level (High/Med./ND)	**OR (95% CI)**	
Methamidophos (High vs ND)	1.64 (0.74, 3.62)	0.73 (0.34, 1.59)
Methamidophos (Med. vs ND)	1.42 (0.66, 3.04)	0.52 (0.23, 1.16)
Trichlorfon (High vs ND)	1.32 (0.59, 2.95)	0.58 (0.25, 1.36)
Trichlorfon (Med. vs ND)	1.35 (0.61, 3.00)	1.22 (0.57, 2.59)
2-level (Detect/ND)	**OR (95% CI)**	
Chlorpyrifos (Detect vs ND)	1.17 (0.59, 2.29)	1.02 (0.52, 2.01)
Phorate (Detect vs ND)	1.66 (0.67, 4.14)	0.80 (0.34, 1.88)

[a] Models adjusted for sex, age at testing, and cord ferritin
High/Medium/ND cut-offs (ng/mL): methamidophos >18.2/1.5-18.2/ND; trichlorfon >1.7/ 0.4-1.7/ND; chlorpyrifos ≥0.04/ND; phorate ≥1.8/ND
*p<0.05

Chapter III Appendix

Table III.A1. Comparison of OP exposures for infants with and without motor data

OP Insecticide	Motor data (n=199)	No motor data (n=39)	Test statistic
Continuous	**Mean (SD)**	**Mean (SD)**	**T (p)**
# OP detects	3.01 (1.65)	2.85 (1.31)	-0.68 (0.50)
Log-naled	0.77 (1.02)	0.56 (0.86)	-1.18 (0.24)
Categorical	**N (%)**	**N (%)**	**X^2 (p)**
Methamidophos			0.47 (0.79)
High	63 (31.7)	14 (35.9)	
Medium	64 (32.2)	13 (33.3)	
<LOD	72 (36.2)	12 (30.8)	
Trichlorfon			0.00 (1.00)
High	51 (25.6)	10 (25.6)	
Medium	51 (25.6)	10 (25.6)	
<LOD	72 (48.7)	19 (48.7)	
Chlorpyrifos			1.00 (0.32)
≥LOD	70 (35.2)	17 (43.6)	
<LOD	129 (64.8)	22 (56.4)	
Phorate			1.59 (0.21)
≥LOD	37 (18.6)	4 (10.3)	
<LOD	162 (81.4)	35 (89.7)	

Table III.A2. Comparison of cord blood pesticide exposures by infant sex

OP Insecticide	Boys (n=171)	Girls (n=165)	Test statistic
Continuous	**Mean (SD)**	**Mean (SD)**	**T (p)**
# OP detects	3.4 (1.8)	3.5 (1.7)	-0.52 (0.61)
Log-naled	2.1 (3.6)	2.3 (3.2)	-0.76 (0.45)
Categorical	**N (%)**	**N (%)**	**X^2 (p)**
Methamidophos			0.27 (0.87)
High	48 (28.1)	46 (27.9)	
Medium	61 (35.7)	63 (38.2)	
<LOD	62 (36.3)	56 (33.9)	
Trichlorfon			0.60 (0.74)
High	62 (36.3)	55 (33.3)	
Medium	34 (19.9)	38 (23.0)	
<LOD	75 (43.9)	72 (43.6)	
Chlorpyrifos			0.03 (0.86)
≥LOD	70 (40.9)	66 (40.0)	
<LOD	101 (59.1)	99 (60.0)	
Phorate			0.11 (0.74)
≥LOD	51 (29.8)	52 (31.5)	
<LOD	120 (70.2)	113 (68.5)	

Table III.A3. Adjusted [a] change/difference in PDMS motor scores at 6 weeks by OP exposure

	Raw Subtest Scores				
OP Insecticide	**Reflexes (n=197)**	**Stationary (n=197)**	**Locomotion (n=197)**	**Grasping (n=197)**	**V-M (n=197)**
Continuous	**β (95% CI) for OP [b]**				
# OP Detects	0.01	0.04	0.03	-0.03	-0.02
	(-0.12, 0.13)	(-0.20, 0.29)	(-0.43, 0.50)	(-0.26, 0.21)	(-0.34, 0.30)
Log-Naled	-0.05	-0.27	-0.03	-0.07	0.03
	(-0.26, 0.15)	(-0.65, 0.11)	(-0.76, 0.70)	(-0.44, 0.29)	(-0.47, 0.53)
3-level (High/Med./ND)	**Difference in least square means (95% CI) [c]**				
Methamidophos	0.00	-0.55	0.06	0.23	0.20
(High vs ND)	(-0.52, 0.52)	(-1.51, 0.40)	(-1.77, 1.89)	(-0.68, 1.15)	(-1.07, 1.46)
Methamidophos	-0.05	-0.14	0.15	0.35	0.02
(Med. vs ND)	(-0.56, 0.47)	(-1.09, 0.81)	(-1.66, 1.96)	(-0.55, 1.26)	(-1.23, 1.27)
Trichlorfon	-0.14	0.24	0.10	-0.32	-0.27
(High vs ND)	(-0.66, 0.38)	(-0.72, 1.20)	(-1.74, 1.93)	(-1.24, 0.60)	(-1.54, 1.00)
Trichlorfon	0.03	-0.05	0.08	-0.35	-0.10
(Med. vs ND)	(-0.48, 0.55)	(-1.00, 0.90)	(-1.74, 1.91)	(-1.26, 0.57)	(-1.36, 1.17)
2-level (Detect/ND)	**Difference in least square means (95% CI) [c]**				
Chlorpyrifos	0.14	-0.07	0.14	0.08	0.27
(Detect vs ND)	(-0.31, 0.58)	(-0.89, 0.74)	(-1.41, 1.70)	(-0.71, 0.86)	(-0.80, 1.34)
Phorate	0.04	0.76	0.18	0.20	-0.11
(Detect vs ND)	(-0.50, 0.59)	(-0.25, 1.77)	(-1.75, 2.12)	(-0.77, 1.17)	(-1.44, 1.22)

[a] Models adjusted for sex, age at testing, and cord ferritin at birth
[b] Estimated change in 6-week motor score per 1 unit increase in OP
[c] Difference in mean 6-week motor score
High/Medium/ND cut-offs (ng/mL): methamidophos >18.2/1.5-18.2/ND; trichlorfon >1.7/ 0.4-1.7/ND; chlorpyrifos ≥0.04/ND; phorate ≥1.8/ND
Abbreviations: V-M, visual-motor integration

Table III.A3, continued

OP Insecticide	Composite Raw Scores GM (n=197)	FM (n=197)	TM (n=197)	Motor Quotients GMQ (n=197)	FMQ (n=197)	TMQ (n=197)
Continuous	**β (95% CI) for OP [b]**					
# OP Detects	0.08 (-0.63, 0.80)	-0.05 (-0.53, 0.44)	0.04 (-0.96, 1.04)	0.00 (-0.55, 0.55)	-0.04 (-0.67, 0.58)	-0.01 (-0.54, 0.52)
Log-Naled	-0.36 (-1.49, 0.77)	-0.04 (-0.81, 0.73)	-0.40 (-1.98, 1.17)	-0.58 (-1.44, 0.29)	0.12 (-0.87, 1.10)	-0.31 (-1.14, 0.53)
3-level (High/Med./ND)	**Difference in least square means (95% CI) [c]**					
Methamidophos (High vs ND)	-0.49 (-3.31, 2.33)	0.43 (-1.50, 2.33)	-0.07 (-4.00, 3.87)	-0.26 (-2.34, 1.82)	0.70 (-1.76, 3.17)	-0.84 (-3.02, 1.34)
Methamidophos (Med. vs ND)	-0.04 (-2.83, 2.75)	0.37 (-1.54, 2.29)	0.34 (-3.56, 4.23)	-0.04 (-2.10, 2.01)	0.51 (-1.93, 2.95)	-0.44 (-2.59, 1.72)
Trichlorfon (High vs ND)	0.20 (-2.63, 3.03)	-0.58 (-2.53, 1.36)	-0.38 (-4.34, 3.58)	-0.06 (-2.23, 2.12)	-0.95 (-3.43, 1.53)	-0.36 (-2.45, 1.73)
Trichlorfon (Med. vs ND)	0.08 (-2.74, 2.89)	-0.43 (-2.37, 1.50)	-0.35 (-4.29, 3.58)	0.05 (-2.12, 2.21)	-1.08 (-3.54, 1.38)	-0.41 (-2.48, 1.67)
2-level (Detect/ND)	**Difference in least square means (95% CI) [c]**					
Chlorpyrifos (Detect vs ND)	0.20 (-2.20, 2.59)	0.34 (-1.29, 1.98)	0.53 (-2.80, 0.86)	0.00 (-1.85, 1.85)	0.29 (-1.82, 2.40)	0.20 (-1.57, 1.97)
Phorate (Detect vs ND)	0.98 (2.00, 3.96)	0.09 (-1.96, 2.14)	1.06 (-3.11, 5.24)	0.65 (-1.65, 2.95)	0.73 (-1.89, 3.34)	0.80 (-1.41, 3.00)

[a] Models adjusted for sex, age at testing, and cord ferritin at birth
[b] Estimated change in 6-week motor score per 1 unit increase in OP
[c] Difference in mean 6-week motor score
High/Medium/ND cut-offs (ng/mL): methamidophos >18.2/1.5-18.2/ND; trichlorfon >1.7/ 0.4-1.7/ND; chlorpyrifos ≥0.04/ND; phorate ≥1.8/ND
Abbreviations: GM, gross motor score; FM, fine motor score; TM, total motor score; GMQ, gross motor quotient; FMQ, fine motor quotient, TMQ, total motor quotient

Table III.A4. Adjusted [a] change/difference in INFANIB motor scores at 9 months by OP exposure

	Subscale scores					Total score
OP insecticide	**Spasticity (n=188)**	**Head/trunk (n=187)**	**Vestibular (n=180)**	**Legs (n=183)**	**French angles (n=184)**	**Total (n=184)**
Continuous	**β (95% CI) for OP [b]**					
# OP Detects	-0.01	0.19 †	0.08	0.02	0.04	0.30
	(-0.10, 0.09)	(-0.01, 0.37)	(-0.25, 0.41)	(-0.15, 0.18)	(-0.23, 0.31)	(-0.30, 0.90)
Log-Naled	0.04	-0.01	0.04	0.21	-0.06	0.16
	(-0.11, 0.18)	(-0.30, 0.28)	(-0.48, 0.57)	(-0.05, 0.46)	(-0.49, 0.36)	(-0.79, 1.10)
3-level (High/Med./ND)	**Difference in least square means (95% CI) [c]**					
Methamidophos	-0.05	-0.03	-0.61	0.54 †	0.28	0.31
(High vs ND)	(-0.41, 0.32)	(-0.74, 0.68)	(-1.90, 0.68)	(-0.08, 1.17)	(-0.78, 1.34)	(-2.03, 2.65)
Methamidophos	-0.02	0.29	0.54	0.50	0.43	1.56
(Med. vs ND)	(-0.38, 0.35)	(-0.41, 0.98)	(-0.74, 1.81)	(-0.13, 1.14)	(-0.62, 1.48)	(-0.77, 3.89)
Trichlorfon	-0.09	0.76 †	0.69	0.00	-0.20	0.81
(High vs ND)	(-0.45, 0.28)	(-0.06, 1.46)	(-0.59, 1.98)	(-0.63, 0.63)	(-1.25, 0.85)	(-1.52, 3.14)
Trichlorfon	-0.03	0.05	0.41	-0.52	0.21	-0.08
(Med. vs ND)	(-0.39, 0.34)	(-0.64, 0.75)	(-0.88, 1.69)	(-1.1, 0.11)	(-0.83, 1.25)	(-2.40, 2.24)
2-level (Detect/ND)	**Difference in least square means (95% CI) [c]**					
Chlorpyrifos	-0.01	-0.08	-0.37	0.16	-0.45	-0.80
(Detect vs ND)	(-0.32, 0.30)	(-0.67, 0.52)	(-1.47, 0.73)	(-0.38, 0.70)	(-1.35, 0.44)	(-2.79, 1.19)
Phorate	-0.02	0.50	0.28	-0.51	-0.25	0.11
(Detect vs ND)	(-0.41, 0.36)	(-0.24, 1.24)	(-1.06, 1.63)	(-1.17, 0.14)	(-1.35, 0.84)	(-2.33, 2.54)

[a] Models adjusted for sex, age at testing, and cord ferritin
[b] Estimated change in 9-month INFANIB score per 1 unit increase in OP
[c] Difference in mean 9-month INFANIB score
High/Medium/ND cut-offs (ng/mL): methamidophos >18.2/1.5-18.2/ND; trichlorfon >1.7/ 0.4-1.7/ND; chlorpyrifos ≥0.04/ND; phorate ≥1.8/ND
†p<0.10

Table III.A5. Comparison of key PDMS-2 variables by infant sex

	6 weeks				9 months						
	Overall (n=199)	Boys (n=105)	Girls (n=94)	Test statistic	Overall		Boys		Girls		Test statistic
PDMS-2 Outcome	Mean (SD)	Mean (SD)	Mean (SD)	T (p)	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	T (p)
Reflexes	2.1	2.1	2.1	-0.4	196	14.0		14.0		14.1	-0.7
	(0.9)	(0.8)	(1.0)	(0.68)		(2.0)	103	(2.1)	93	(1.8)	(0.50)
Stationary	3.7	3.6	3.9	-0.8	202	33.2		33.0		33.4	-0.8
	(2.5)	(2.4)	(2.5)	(0.44)		(3.1)	104	(3.2)	98	(3.0)	(0.42)
Locomotion	6.3	6.4	6.3	0.6	201	37.1		37.1		37.0	0.2
	(0.9)	(0.1)	(0.1)	(0.53)		(7.9)	103	(7.4)	98	(8.4)	(0.88)
Grasping	3.3	3.3	3.3	0.0	205	36.1		35.9		36.3	-1.0
	(1.9)	(1.8)	(2.0)	(0.99)		(3.3)	106	(3.3)	99	(3.2)	(0.34)
V-M	3.9	3.8	3.9	-0.5	206	45.5		44.8		46.2	-1.9
	(1.3)	(1.3)	(1.3)	(0.64)		(5.2)	106	(4.5)	100	(5.7)	(0.06)
GM	12.2	12.0	12.3	-0.5	194	84.4		84.2		84.7	-0.3
	(3.1)	(2.9)	(3.3)	(0.59)		(11.7)	101	(11.2)	93	(12.3)	(0.79)
FM	7.2	7.2	7.3	-0.2	205	81.6		80.7		82.5	-1.6
	(2.6)	(2.3)	(2.8)	(0.80)		(7.7)	106	(7.2)	99	(8.1)	(0.11)
TM	19.4	19.2	19.5	-0.48	193	166.1		164.9		167.3	-1.0
	(4.7)	(4.1)	(5.4)	(0.63)		(16.4)	101	(15.2)	92	(17.6)	(0.30)

Abbreviations: V-M, visual-motor integration; GM, gross motor score; FM, fine motor score; TM, total motor score; GMQ, gross motor quotient; FMQ, fine motor quotient, TMQ, total motor quotient

Table III.A6. Sex-stratified change/difference in PDMS motor scores at 6 weeks by OP exposure [a]

	Raw Subtest Scores for BOYS (n=103)				
OP Insecticide	**Reflexes**	**Stationary**	**Locomotion**	**Grasping**	**V-M**
Continuous	**β (95% CI) for OP [b]**				
# OP Detects	0.06 (-0.14, 0.25)	0.05 (-0.30, 0.40)	-0.03 (-0.68, 0.61)	0.02 (-0.32, 0.36)	-0.04 (-0.46, 0.37)
Log-Naled	0.03 (-0.29, 0.34)	-0.22 (-0.79, 0.35)	0.09 (-0.95, 1.14)	0.23 (-0.32, 0.77)	0.05 (-0.62, 0.72)
3-level (High/Med./ND)	**Difference in least square means (95% CI) [c]**				
Methamidophos (High vs ND)	-0.07 (-0.83, 0.69)	-0.55 (-1.94, 0.84)	0.21 (-2.35, 2.77)	0.97 (-0.35, 2.29)	0.36 (-1.27, 1.99)
Methamidophos (Med. vs ND)	-0.12 (-0.84, 0.59)	-0.47 (-1.77, 0.83)	0.08 (-2.32, 2.47)	0.64 (-0.60, 1.88)	-0.28 (-1.80, 1.24)
Trichlorfon (High vs ND)	-0.05 (-0.83, 0.73)	0.39 (-1.03, 1.80)	-0.31 (-2.89, 2.27)	-0.33 (-1.69, 1.03)	-0.21 (-1.88, 1.47)
Trichlorfon (Med. vs ND)	0.29 (-0.43, 1.02)	0.34 (-0.98, 1.65)	0.25 (-2.14, 2.65)	-0.29 (-1.55, 0.97)	0.04 (-1.51, 1.60)
2-level (Detect/ND)	**Difference in least square means (95% CI) [c]**				
Chlorpyrifos (Detect vs ND)	0.40 (-0.23, 1.04)	0.15 (-1.02, 1.33)	-0.08 (-2.23, 2.07)	-0.18 (-1.30, 0.93)	0.18 (-1.17, 1.52)
Phorate (Detect vs ND)	0.07 (-0.73, 0.88)	0.42 (-1.02, 1.86)	-0.08 (-2.74, 2.58)	-0.14 (-1.53, 1.25)	-0.07 (-1.77, 1.63)

	Raw Subtest Scores for GIRLS (n=94)				
OP Insecticide	**Reflexes**	**Stationary**	**Locomotion**	**Grasping**	**V-M**
Continuous	**β (95% CI) for OP [b]**				
# OP Detects	-0.05 (-2.33, 0.13)	0.06 (-0.28, 0.40)	0.06 (-0.62-0.73)	-0.10 (-0.43-0.23)	-0.04 (-0.54, 0.45)
Log-Naled	-0.11 (-0.39, 0.16)	-0.29 (-0.82, 0.23)	-0.16 (-1.21-0.89)	-0.36 (-0.86-0.14)	0.00 (-0.75, 0.75)
3-level (High/Med./ND)	**Difference in least square means (95% CI) [c]**				
Methamidophos (High vs ND)	0.04 (-0.68, 0.76)	-0.46 (-1.82, 0.90)	-0.25 (-2.95, 2.46)	-0.60 (-1.89, 0.70)	-0.00 (-1.98, 1.98)
Methamidophos (Med. vs ND)	0.08 (-0.66, 0.83)	0.30 (-1.11, 1.71)	0.28 (-2.52, 3.08)	-0.02 (-1.37, 1.32)	0.39 (-1.66, 2.44)
Trichlorfon (High vs ND)	-0.27 (-0.98, 0.44)	0.15 (-1.20, 1.50)	0.28 (-2.40, 2.96)	-0.39 (-1.66, 0.89)	-0.52 (-2.48, 1.44)
Trichlorfon (Med. vs ND)	-0.36 (-1.11, 0.40)	-0.46 (-1.89, 0.97)	-0.20 (-3.05, 2.64)	-0.52 (-1.87, 0.83)	-0.56 (-2.64, 1.52)
2-level (Detect/ND)	**Difference in least square means (95% CI) [c]**				
Chlorpyrifos (Detect vs ND)	-0.16 (-0.78, 0.45)	-0.34 (-1.50-0.82)	0.29 (-2.01, 2.58)	0.36 (-0.77, 1.48)	0.43 (-1.25, 2.11)
Phorate (Detect vs ND)	-0.05 (-0.82, 0.71)	1.22 † (-0.22, 2.66)	0.31 (-2.57, 3.19)	0.42 (-0.97, 1.82)	-0.43 (-2.53, 1.67)

[a] Models adjusted for age at testing and cord ferritin
[b] Estimated change in 6-week PDMS score per 1 unit increase in OP
[c] Difference in mean 6-week PDMS score
†p<0.10
High/Medium/ND cut-offs (ng/mL): methamidophos >18.2/1.5-18.2/ND; trichlorfon >1.7/ 0.4-1.7/ND; chlorpyrifos ≥0.04/ND; phorate ≥1.8/ND
Abbreviations: V-M, visual-motor integration

Table III.A6, continued

	Composite Raw Scores for BOYS (n=103)		
OP Insecticide	**GM**	**FM**	**TM**
Continuous	**β (95% CI) for OP [b]**		
# OP Detects	0.08	-0.02	0.06
	(-0.92, 1.07)	(-0.70, 0.65)	(-1.29, 1.40)
Log-Naled	-0.11	0.28	0.17
	(-1.73, 1.50)	(-0.81, 1.37)	(-2.00, 2.34)
3-level (High/Med./ND)	**Difference in least square means (95% CI) [c]**		
Methamidophos (High vs ND)	-0.42 (-4.36, 3.52)	1.34 (-1.31, 3.98)	0.95 (-4.35, 6.25)
Methamidophos (Med. vs ND)	-0.53 (-4.22, 3.16)	0.36 (-2.12, 2.83)	-0.18 (-5.13, 4.78)
Trichlorfon (High vs ND)	0.10 (-3.88, 4.08)	-0.53 (-3.24, 2.19)	-0.40 (-5.77, 4.97)
Trichlorfon (Med. vs ND)	0.89 (-2.80, 4.58)	-0.24 (-2.76, 2.29)	0.68 (-4.30, 5.66)
2-level (Detect/ND)	**Difference in least square means (95% CI) [c]**		
Chlorpyrifos (Detect vs ND)	0.46 (-2.85, 3.77)	-0.01 (-2.22, 2.19)	0.44 (-3.98, 4.86)
Phorate (Detect vs ND)	0.44 (-3.64, 4.52)	-0.20 (-2.98, 2.58)	0.26 (-5.27, 5.78)
	Composite Raw Scores for GIRLS (n=94)		
OP Insecticide	**GM**	**FM**	**TM**
Continuous	**β (95% CI) for OP [b]**		
# OP Detects	0.05	-0.14	-0.08
	(-0.99, 1.10)	(-0.86, 0.58)	(-1.60, 1.43)
Log-Naled	-0.57	-0.36	-0.92
	(-2.19, 1.06)	(-1.45, 0.73)	(-3.25, 1.42)
3-level (High/Med./ND)	**Difference in least square means (95% CI) [c]**		
Methamidophos (High vs ND)	-0.65 (-4.83, 3.53)	-0.60 (-3.47, 2.26)	-1.25 (-7.25, 4.75)
Methamidophos (Med. vs ND)	0.67 (-3.67, 5.00)	0.36 (-2.61, 3.33)	1.02 (-5.20, 7.25)
Trichlorfon (High vs ND)	0.12 (-4.03, 4.28)	-0.90 (-3.73, 1.94)	-0.75 (-6.75, 5.25)
Trichlorfon (Med. vs ND)	-1.07 (-5.43, 3.39)	-1.08 (-4.09, 1.94)	-2.11 (-8.48, 4.26)
2-level (Detect/ND)	**Difference in least square means (95% CI) [c]**		
Chlorpyrifos (Detect vs ND)	-0.19 (-3.73, 3.36)	0.79 (-1.67, 3.24)	0.60 (-4.52, 5.71)
Phorate (Detect vs ND)	1.44 (-3.02, 5.89)	-0.00 (-3.08, 3.07)	1.50 (-4.97, 7.96)

[a] Models adjusted for age at testing and cord ferritin
[b] Estimated change in 6-week PDMS score per 1 unit increase in OP
[c] Difference in mean 6-week PDMS score
†p<0.10
High/Medium/ND cut-offs (ng/mL): methamidophos >18.2/1.5-18.2/ND; trichlorfon >1.7/ 0.4-1.7/ND; chlorpyrifos ≥0.04/ND; phorate ≥1.8/ND
Abbreviations: GM, gross motor score; FM, fine motor score; TM, total motor score

Table III.A6, continued

	Motor Quotients for BOYS (n=103)		
OP Insecticide	**GMQ**	**FMQ**	**TMQ**
Continuous	**β (95% CI) for OP [b]**		
# OP Detects	0.13 (-0.66, 0.93)	0.07 (-0.81, 0.94)	0.11 (-0.61, 0.84)
Log-Naled	-0.38 (-1.67, 0.91)	0.64 (-0.76, 2.04)	0.00 (-1.17, 1.18)
3-level (High/Med./ND)	**Difference in least square means (95% CI) [c]**		
Methamidophos (High vs ND)	0.64 (-2.22, 3.49)	3.00 † (-0.40, 6.39)	-0.93 (-4.08, 2.21)
Methamidophos (Med. vs ND)	-0.71 (-2.75, 3.61)	0.18 (-2.99, 3.36)	-1.21 (-4.16, 1.73)
Trichlorfon (High vs ND)	0.43 (-2.75, 3.61)	-0.36 (-3.86, 3.14)	0.23 (-2.67, 3.13)
Trichlorfon (Med. vs ND)	1.29 (-1.66, 4.24)	-0.27 (-3.52, 2.98)	0.66 (-2.03, 3.35)
2-level (Detect/ND)	**Difference in least square means (95% CI) [c]**		
Chlorpyrifos (Detect vs ND)	0.90 (-1.75, 3.54)	-0.45 (-3.33, 2.43)	0.34 (-2.06, 2.74)
Phorate (Detect vs ND)	0.66 (-2.61, 3.93)	0.60 (-2.99, 4.19)	0.75 (-2.22, 3.73)
	Motor Quotients for GIRLS (n=94)		
OP Insecticide	**GMQ**	**FMQ**	**TMQ**
Continuous	**β (95% CI) for OP [b]**		
# OP Detects	-0.12 (-0.89, 0.66)	-0.19 (-1.11, 0.73)	-0.14 (-0.92, 0.65)
Log-Naled	-0.73 (-1.94, 1.67)	-0.36 (-1.78, 1.05)	-0.55 (-1.77, 0.67)
3-level (High/Med./ND)	**Difference in least square means (95% CI) [c]**		
Methamidophos (High vs ND)	-1.01 (-4.15, 2.12)	-1.50 (-5.14, 2.15)	-0.64 (-3.78, 2.49)
Methamidophos (Med. vs ND)	0.78 (-2.47, 4.03)	0.81 (-2.97, 4.58)	0.58 (-2.67, 3.83)
Trichlorfon (High vs ND)	-0.53 (-3.62, 2.56)	-1.64 (-5.23, 1.95)	-0.93 (-4.04, 2.18)
Trichlorfon (Med. vs ND)	-1.40 (-4.68, 1.88)	-2.37 (-6.19, 1.44)	-1.80 (-5.11, 1.50)
2-level (Detect/ND)	**Difference in least square means (95% CI) [c]**		
Chlorpyrifos (Detect vs ND)	-0.97 (-3.60, 1.67)	1.18 (-1.97, 4.33)	0.05 (-2.63, 2.72)
Phorate (Detect vs ND)	0.70 (-2.62, 4.02)	0.73 (-3.19, 4.66)	0.90 (-2.46, 4.27)

[a] Models adjusted for age at testing and cord ferritin
[b] Estimated change in 6-week PDMS score per 1 unit increase in OP
[c] Difference in mean 6-week PDMS score
†p<0.10
High/Medium/ND cut-offs (ng/mL): methamidophos >18.2/1.5-18.2/ND; trichlorfon >1.7/ 0.4-1.7/ND; chlorpyrifos ≥0.04/ND; phorate ≥1.8/ND
Abbreviations: GMQ, gross motor quotient; FMQ, fine motor quotient, TMQ, total motor quotient

Table III.A7. Comparison of INFANIB variables by infant sex

	6 weeks				9 months						
	Overall (n=199)	Boys (n=105)	Girls (n=94)	Test statistic	Overall		Boys		Girls		Test statistic
INFANIB Outcome	Mean (SD)	Mean (SD)	Mean (SD)	T (p)	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	T (p)
Spasticity	18.5 (1.4)	18.5 (1.3)	18.6 (1.5)	-0.3 (0.74)	203	19.9 (0.5)	106	19.9 (0.4)	97	19.9 (0.6)	0.7 (0.48)
Head/trunk	14.3 (1.4)	14.3 (1.4)	14.4 (1.3)	-0.5 (0.59)	202	16.5 (2.4)	107	16.5 (2.6)	95	16.4 (2.3)	0.0 (0.98)
Vestibular					195	17.3 (3.5)	103	17.3 (3.4)	92	17.4 (3.7)	-0.1 (0.90)
Legs	13.4 (1.6)	13.5 (1.5)	13.3 (1.7)	0.9 (0.39)	198	15.0 (1.9)	102	15.0 (1.8)	96	15.0 (2.1)	-0.1 (0.94)
French angles	16.1 (3.1)	16.4 (2.8)	15.8 (3.4)	1.4 (0.16)	199	17.3 (2.8)	102	17.2 (2.8)	97	17.3 (2.9)	-0.2 (0.87)
Total	62. 4 (4.3)	62.7 (4.09)	62.0 (4.5)	1.1 (0.28)	199	85.5 (8.2)	105	85.2 (8.3)	94	85.9 (8.1)	-0.5 (0.59)

Table III.A8. Sex-stratified change/difference in INFANIB motor scores at 9 months by OP exposure [a]

	Subscale scores for BOYS					Total score for BOYS
OP Insecticide	**Spasticity (n=106)**	**Head/trunk (n=107)**	**Vestibular (n=103)**	**Legs (n=102)**	**French angles (n=102)**	**Total (n=105)**
Continuous	**β (95% CI) for OP [b]**					
# OP Detects	0.00 (-0.13, 0.14)	0.36 * (0.08, 0.65)	0.30 (-0.19, 0.79)	0.00 (-0.23, 0.23)	0.07 (-0.32, 0.46)	0.75 † (-0.14, 1.65)
Log-Naled	0.05 (-0.15, 0.25)	0.00 (-0.43, 0.44)	-0.03 (-0.77, 0.71)	-0.15 (-0.51, 0.20)	0.23 (-0.36, 0.82)	0.14 (-1.22, 1.51)
3-level (High/Med./ND)	**Difference in least square means (95% CI) [c]**					
Methamidophos (High vs ND)	0.05 (-0.44, 0.53)	0.09 (-0.95, 1.13)	0.06 (-1.72, 1.84)	0.35 (-0.48, 1.18)	0.66 (-0.76, 2.09)	1.42 (-1.85, 4.69)
Methamidophos (Med. vs ND)	0.05 (-0.40, 0.50)	0.02 (-0.95, 0.99)	0.77 (-0.88, 2.42)	0.49 (-0.32, 1.29)	0.08 (-1.25, 1.42)	1.23 (-1.86, 4.32)
Trichlorfon (High vs ND)	-0.00 (-0.48, 0.47)	1.16 * (0.12, 2.19)	1.73 † (-0.07, 3.54)	0.24 (-0.60, 1.09)	-0.05 (-1.49, 1.39)	2.47 (-0.83, 5.77)
Trichlorfon (Med. vs ND)	0.00 (-0.46, 0.46)	0.47 (-0.52, 1.46)	0.72 (-0.97, 2.41)	-0.43 (-1.24, 0.38)	-0.17 (-1.53, 1.20)	0.78 (-2.38, 3.94)
2-level (Detect/ND)	**Difference in least square means (95% CI) [c]**					
Chlorpyrifos (Detect vs ND)	-0.05 (-0.46, 0.35)	0.17 (-0.71, 1.04)	-0.41 (-1.92, 1.10)	0.06 (-0.67, 0.79)	-0.33 (-1.53, 0.87)	-0.84 (-3.63, 1.94)
Phorate (Detect vs ND)	-0.08 (0.60, 0.44)	0.77 (-0.36, 1.90)	0.25 (-1.71, 2.22)	-0.56 (-1.46, 0.35)	0.10 (-1.44, 1.63)	0.77 (-2.80, 4.34)

[a] Models adjusted for age at testing and cord ferritin
[b] Estimated change in 9-month INFANIB score per 1 unit increase in OP
[c] Difference in mean 9-month INFANIB score
†p<0.10, , *p<0.05
High/Medium/ND cut-offs (ng/mL): methamidophos >18.2/1.5-18.2/ND; trichlorfon >1.7/ 0.4-1.7/ND; chlorpyrifos ≥0.04/ND; phorate ≥1.8/ND

Table III.A8, continued

	Subscale scores for GIRLS					Total score for GIRLS
OP Insecticide	**Spasticity (n=97)**	**Head/trunk (n=95)**	**Vestibular (n=92)**	**Legs (n=96)**	**French angles (n=97)**	**Total (n=94)**
Continuous	**β (95% CI) for OP [b]**					
# OP Detects	-0.01	0.07	-0.09	0.05	-0.00	-0.09
	(-0.15, 0.13)	(-0.16, 0.31)	(-0.56, 0.38)	(-0.18, 0.28)	(-0.39, 0.38)	(-0.92, 0.75)
Log-Naled	0.02	-0.00	0.11	0.51 **	-0.35	0.11
	(-0.20, 0.25)	(-0.39, 0.38)	(-0.66, 0.88)	(0.14, 0.88)	(-0.98 0.28)	(-1.24, 1.46)
3-level (High/Med./ND)	**Difference in least square means (95% CI) [c]**					
Methamidophos	-0.12	0.04	-1.32	0.81	-0.18	-0.77
(High vs ND)	(-0.70, 0.47)	(-0.95, 1.02)	(-3.31, 0.66)	(-0.17, 1.78)	(-1.82, 1.45)	(-4.28, 2.73)
Methamidophos	-0.10	0.69	0.18	0.57	0.79	1.91
(Med. vs ND)	(-0.70, 0.51)	(-0.32, 1.69)	(-1.89, 2.26)	(-0.45, 1.58)	(-0.90, 2.48)	(-1.74, 5.56)
Trichlorfon	-0.16	0.46	-0.24	-0.10	-0.37	-0.81
(High vs ND)	(-0.73, 0.41)	(-0.49, 1.41)	(-2.19, 1.71)	(-1.06, 0.86)	(-1.97, 1.22)	(-4.23, 2.61)
Trichlorfon	-0.08	-0.49	0.20	-0.53	0.81	-1.11
(Med. vs ND)	(-0.68, 0.51)	(-1.48, 0.50)	(-1.89, 2.28)	(-1.53,0.47)	(-0.85, 2.48)	(-4.71, 2.48)
2-level (Detect/ND)	**Difference in least square means (95% CI) [c]**					
Chlorpyrifos	0.04	-0.39	-0.34	0.27	-0.63	-0.97
(Detect vs ND)	(-0.45, 0.53)	(-1.20, 0.42)	(-2.01, 1.34)	(-0.56, 1.09)	(-1.99, 0.74)	(-3.89, 1.96)
Phorate	0.05	0.43	0.35	-0.37	-0.69	-0.39
(Detect vs ND)	(-0.53, 0.64)	(-0.55, 1.41)	(-1.61, 2.30)	(-1.34, 0.60)	(-2.32, 0.93)	(-3.83, 3.05)

[a] Models adjusted for age at testing and cord ferritin
[b] Estimated change in 9-month INFANIB score per 1 unit increase in OP
[c] Difference in mean 9-month INFANIB score
†p<0.10, , *p<0.05, , **p<0.01
High/Medium/ND cut-offs (ng/mL): methamidophos >18.2/1.5-18.2/ND; trichlorfon >1.7/ 0.4-1.7/ND; chlorpyrifos ≥0.04/ND; phorate ≥1.8/ND

CHAPTER IV

AIM 3: Prenatal exposure to organophosphate insecticides and infant sensory function

Abstract

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

Methods: 30 OPs were measured in umbilical cord blood using gas chromatography tandem mass spectrometry in a cohort of Chinese infants. Grating visual acuity (VA) (n=198) and auditory brainstem response (ABR) (n=181) were assessed at 6 weeks, 9 months, and 18 months. Outcomes included VA score and ABR wave V latency, central conduction time ([CCT], wave V - wave I latencies), and the III-V and I-III inter-peak intervals ([IPI] wave V- wave III and wave III- wave I latencies, respectively).

Results: Prenatal chlorpyrifos exposure was associated with lower 9-month grating VA scores; scores were 0.64 (95% CI: -1.22, -0.06) points lower for exposed versus unexposed infants (p=0.03). None of the OPs examined were associated with infant ABR latencies, though chlorpyrifos and phorate were both

significantly inversely associated with head circumference (HC) at 9 months; HCs were 0.41 (95% CI: 0.75, 0.6) cm and 0.44 (95% CI: 0.88, 0.1) cm smaller for chlorpyrifos (p=0.02) and phorate (p=0.04), respectively.

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

Introduction

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

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

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

OPs have also emerged as a concern for developmental neurotoxicity, even at relatively low-levels of exposure where cholinergic toxicity would not be

present. Due to concerns of early-life neurotoxicity, a number of commonly used OPs have been banned for residential uses (U.S.EPA, 2011; W. Zhang, et al., 2011). Rapidly developing fetal brains may be susceptible to possible long-term effects of prenatal OP exposure (Garcia, Seidler, & Slotkin, 2005). Studies of prenatal exposure to OPs provide evidence of associations with neurological effects in childhood such as IQ deficits (Bouchard, et al., 2011; Engel, et al., 2011; V. Rauh, et al., 2011) and cognitive delays (Bouchard, et al., 2011; Engel, et al., 2011; Eskenazi, et al., 2007; V. Rauh, et al., 2011; V. A. Rauh, et al., 2006), as well as increased diagnoses of autism (Shelton, et al., 2014), attention deficit-hyperactivity (Marks, et al., 2010; V. A. Rauh, et al., 2006), and pervasive developmental (Eskenazi, et al., 2007; V. A. Rauh, et al., 2006) disorders.

Despite a growing body of evidence regarding early-life OP exposure and these commonly studied neurodevelopmental and cognitive endpoints, much less is known about how exposure to OPs may affect childhood sensory functions, such as visual and auditory function. Proper visual and auditory system development in infancy is crucial to later learning processes such as the development of language and other forms of communication, as well providing the foundation for reading skills in childhood (Algarin, Peirano, Garrido, Pizarro, & Lozoff, 2003; Chonchaiya, et al., 2013). Only two epidemiological studies to date have examined prenatal OP exposure and either visual or auditory-related outcomes (Handal, Harlow, Breilh, & Lozoff, 2008; Sturza, et al., 2016). Maternal self-reported occupational OP exposure during pregnancy was associated with significantly higher odds of poor visual acuity in infants (Handal, et al., 2008),

while number of pesticides (OPs and other classes) in cord blood was associated with slower auditory signal transmission in infants (Sturza, et al., 2016). These studies provide some preliminary evidence that prenatal OP exposure may negatively affect early-childhood sensory-related functions.

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

Methods

Ethics Statement

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

Study Sample

Pregnant women were recruited late in gestation (37-42 weeks) from Fuyang Maternal and Children's hospital between 2008 and 2011. 359 women with healthy, uncomplicated, singleton pregnancies were enrolled into a longitudinal study of iron deficiency and infant neurodevelopment. 237 women had a sufficient volume of cord blood available for pesticide analysis. Infant development was assessed at three follow-up visits around 6 weeks, 9 months, and 18 months of age.

Organophosphate Insecticides (OPs)

The protocol for the determination of pesticides in cord blood has been described elsewhere (Silver, Shao, et al., 2016). Briefly, cord blood plasma samples were analyzed for 24 OPs and 6 OP metabolites at the Institute of Toxicology at Nanjing Medical University using gas chromatography tandem mass spectrometry (GC-MS/MS) (Perez, et al., 2010; ThermoScientific). Limits of detection (LODs) were determined by analyzing fortified serum on a signal-to-noise (S/N) ratio of three. Quality control samples were generated using plasma samples and a known amount of OP standard (0.675 or 1.35 ng/mL). Quality control samples and blanks were analyzed concurrently with samples.

Individual OPs were treated as continuous variables when detection rates were ≥80% (values <LOD were replaced with LOD/√2), three-level ordinal (<LOD, medium, high [median split for those above LOD]) when detection rates were 40-79%, and dichotomous (<LOD/detect), when detection rates were 10-39%. Naled (99.6% detected) was log-transformed prior to statistical analysis to account for its right-skewed distribution; methamidophos (64.6% detected) and trichlorfon (51.0% detected) were converted to 3-level ordinal variables; chlorpyrifos (36.7% detected) and phorate (16.9% detected) were treated as dichotomous. A "number of OP detects" variable was created by assigning OPs <LOD a value of 0 and detects a value of 1; these were then summed to create an index of OP exposure for each infant (Wickerham, et al., 2012).

Grating Visual Acuity (VA)

Grating VA was estimated here using the Teller acuity card (TAC) preferential looking procedure. This test provides a quantitative measure of

binocular grating acuity for infants and nonverbal children. Grating VA improves throughout in infancy and childhood with the maturation of the visual pathway in the developing brain (Tau & Peterson, 2010).

Grating VA was measured at three time points, 6 weeks, 9 months, and 18 months using a TAC procedure. The ambient lighting luminance was 85 candelas/m^2. Examiners were blinded to infant exposure status. Infants faced a TAC test stage (38 cm away) and were held upright by their mothers. Examiners presented a series of mounted prints, with black and white vertical gratings to one side and a gray blank on the other side, through a rectangular opening in the test stage. Gratings ranged from coarse to fine (0.44-27 cycles/degree) and cards had 35% reflectance. Cards were presented in descending order, with wider (coarse) gratings presented first. Gratings were presented on both the left and right sides of the print to avoid habituation. Examiners observed the infants' looking behavior through a small central aperture in the test stage and determined which cards the infants could see. Examiners repeated the presentation several times until a confident judgment could be made based on consistent looking toward the location of the grating. Grating VA score was estimated as the spatial frequency of the finest grating that the infant could resolve. If the tester was uncertain about the acuity estimate, a second examiner (blinded to the results of the first testing) re-tested the infant. If the infant was uncooperative, parents were asked to return for testing another day. Grating VA data was available for 196 infants at 6-week testing, 200 infants at 9-month testing, and 179 infants at 18-month testing.

Auditory Brainstem Response (ABR)

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

ABR was measured in 6-week-, 9-month-, and 18-month-old infants during unsedated sleep using a Biologic Navigator (Bio-Logic Systems Corp., Mundelein, IL)/Traveler evoked potential system. Infants first underwent a standard hearing screening protocol. Stimuli for the hearing screening test were a series of square wave rarefaction monophasic clicks delivered to both ears by insert transducers with a presentation rate of 31.3/second, a duration of 100 μs, and an intensity of 30 dB, nHL. Infants who passed the hearing screening continued on to the ABR protocol. Stimuli for the ABR test were also square wave rarefaction monophasic clicks delivered to each ear by insert transducers with a presentation rate of 11.7/second, a duration of 100 μs, and an intensity of 80 dB, nHL. The recording epoch was 74.67 ms. ABRs were recorded by silver/silver chloride electrodes attached to infant's forehead in three locations: midline below the hairline (non-inverting), mastoid on ipsilateral mastoid

(inverting) and contralateral mastoid (ground). The impedance was <10 kΩ. The program rejected ABR traces contaminated by high-amplitude artifacts (voltage >±23.80 μV). 1300 sweeps were averaged for each run and two succesive averages were obtained for each ear (2600 sweeps). Right and left ears were averaged (5200 sweeps) to obtain a single measurement for each infant.

ABR outcomes included wave V latency, central conduction time ([CCT], wave V latency – wave I latency), and the III-V and I-III inter-peak intervals ([IPI] wave V latency – wave III latency and wave III latency – wave I latency, respectively). Wave V ABR latencies were available for 183 infants at 6-week testing, 176 infants at 9-month testing, and 139 infants at 18-month testing. Other ABR data (waves I and III) was available for 182, 154, and 106 infants for the 6-week, 9-month and 18-month time points, respectively.

Covariates

Sex was recorded at the time of birth. Cord blood iron status was defined using serum ferritin, which was measured using chemiluminescent immunoassay (IMMULITE, Diagnostic Products) and categorized into deficient or sufficient (≤75 and >75 μg/L). Serum ferritin values >370 μg/L were excluded due to the possibility of infection or inflammation. Infant age was recorded at time of 6-week, 9-month, and 18-month testing. Maternal education, occupation, and family income were obtained by maternal self-report from a family background questionnaire administered at the 6-week follow-up visit. Season of testing was determined categorizing the month of the developmental testing into spring (March-May), summer (June-September), or fall/winter (October-February).

Head circumference was measured at the 6-week, 9-month, and 18-month follow-up visits using a soft plastic tape placed just above the eyebrows and wrapped around the widest part of the head.

Statistical analysis

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

Linear mixed models (LMM) with random intercepts were used to evaluate associations between cord OP exposures and either grating VA scores or ABR outcomes (wave V latency, CCT, IPI I-III, IPI III-V) at 6 weeks, 9 months, and 18 months. Given our small sample sizes, especially for the 18-month time point, we took a conservative approach to choosing covariates for our models. Sex, age at testing, and cord ferritin were chosen *a priori*. Additional covariates considered for inclusion were maternal education and occupation, income, and season in which neurological testing took place. Bivariate analyses revealed that season of testing was the only variable with the potential to be a true confounder, since it was associated with both OP exposure and the outcomes. However, inclusion of season in the models did not significantly influence the results. Therefore, to maximize our power, final models were minimally adjusted for sex, age at testing, and cord ferritin. To enable comparisons of effect estimates at

each of the three time points, we included “time” as a class variable and “time*OP” in our LMM models. We used the “lsmeans” procedure to compare estimates for the categorical OP exposures. For continuous exposures (number of OP detects, log-naled), the parameter of interest was the slope estimating change in 6-week, 9-month or 18-month VA score or ABR latencies per 1 unit increase in OP. For categorical exposures (methamidophos, trichlorfon, chlorpyrifos, phorate), the parameter of interest was the difference in mean 6-week, 9-month or 18-month VA score or ABR latencies by category of OP exposure.

To examine for sex dimorphic effects we ran our LMM models stratified by infant sex. We additionally examined the potential effects of iron as an effect modifier by stratifying by cord iron status (sufficient/deficient). Iron deficiency was previously found to strengthen associations between overall pesticide exposure and ABR latencies (Sturza, et al., 2016). We also explored the hypothesis that prenatal exposure might be associated with reduced head circumference (Berkowitz, et al., 2004). Head circumference may be directly associated with auditory pathway length and has been used as a rough proxy for pathway length in previous studies of lead exposure and ABR (Rothenberg, Poblano, & Schnaas, 2000; Silver, Li, et al., 2016). Therefore, we explored associations between prenatal OP exposure and head circumference at our three time points, as well as head circumference as a confounder of our OP/ABR analyses.

Results

5 OPs were detected in ≥10% of cord blood samples: naled, methamidophos, trichlorfon, chlorpyrifos, and phorate. Distributions of the detectable OPs in cord blood and their LODs have been reported previously Table III.2). There were no underlying differences in OP exposure among those with and without sensory data at 6 weeks or 9 months (Table IV.A1). Many infants were missing ABR wave I and III data at 18 months (n=132); methamidophos exposure was significantly different among infants with and without 18 month ABR data (Table IV.A1). We previously reported that there were no significant differences in cord blood pesticides by infant sex (Table III.A2).

Sample characteristics are shown in Table IV.1. The mean (standard deviation) of OP detects per sample was 3.0 (1.6). Infants all had healthy birth weights and were carried to full-term. Additional characteristics of the study population have been reported previously (Silver, Shao, et al., 2016).

Adjusted LMM results for grating VA score are shown in Table IV.2. In general, 9-month grating VA scores were lower for most of the cord OP exposures, though only chlorpyrifos was statistically significant. Infants with prenatal exposure to chlorpyrifos had 9-month grating VA scores that were, on average, 0.64 points lower than unexposed infants (p=0.03). Prenatal OP exposure was not significantly associated with VA scores at 6 weeks or 18 months (Table IV.2).

Adjusted LMM results for the ABR outcomes are shown in Table IV.3. The results are largely null. ABR latencies are slightly longer (indicating slower auditory signal transmission) for infants with high prenatal methamidophos exposure, compared to unexposed infants, across all ABR outcomes at all three time points, though these differences were not statistically significant (Table IV.3).

Bivariate analyses revealed that 6-week grating VA scores differed significantly by infant sex (Table IV.4). 6-week scores were lower in males compared to females; means (SD) were 1.07 (0.37) for boys and 1.28 (0.68) for girls (p=0.01). VA scores did not significantly differ by sex at the other time points (Table IV.4). Sex-stratified LMM results for OP/grating VA associations are shown in Figure IV.1. There are no noticeable differences by sex at the early time points, however by 18 months, differences start to emerge for some of the OP exposures (OP detects, methamidophos, chlopryrifos and phorate). For these four OP exposures, VA scores appeared to be consistently lower in exposed girls and consistently higher in exposed boys. For example, 18-month VA scores were 0.94 points lower for chlorpyrifos-exposed girls, compared to unexposed (p=0.08), while scores were 0.68 points higher for exposed boys, compared to unexposed (p=0.08) (Figure IV.1).

Bivariate analyses similarly revealed that ABR scores differed significantly by infant sex at all three time points (Table IV.5). Wave V and CCT latencies were consistently higher in girls compared to boys. For example, at 6 weeks, Wave V means (SD) were 6.45 (0.26) ms for boys and 6.55 (0.27) ms for girls

(p<0.0001). Similar effects were seen for the other time points (Table IV.5). Sex-stratified LMM results for OP/ABR associations were largely inconclusive and are shown in Figure IV.2 (6 weeks) and Table IV.A2 (9 and 18 months). Differences by sex seemed to be the most pronounced at 6 weeks, though the only consistent sex-specific differences across ABR outcomes were observed for naled at 6-weeks. Estimates were -0.011, -0.003, -0.003, and -0.001 for boys for wave V, CCT, IPI III-V and IPI I-III, respectively, while for girls they were 0.017, 0.030, 0.014, and 0.016, respectively (Figure IV.2).

Further analysis of our ABR models stratified by cord iron status (sufficient/deficient) did not show any significant evidence of effect modification by iron in our sample (Table IV.A3). Our exploration of head circumference revealed that infants prenatally exposed to chlorpyrifos and phorate had reduced head circumferences at 9 months, compared to unexposed infants (Table IV.6). Head circumferences were 0.41 (95% CI: 0.75, 0.6) cm and 0.44 (95% CI: 0.88, 0.1) cm smaller in infants exposed to chlorpyrifos (p=0.02) and phorate (p=0.04), respectively, compared to unexposed. However, despite evidence of these associations, we did not find any confounding by head circumference when we added it to our OP/ABR models (Table IV.A4).

Discussion

Here we found that infants prenatally exposed to chlorpyrifos had lower grating VA scores at 9 months, compared to unexposed infants. None of the

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

To our knowledge there are only two previous studies that have examined the effects of prenatal OP exposure on visual- or auditory-related functions in infancy or childhood. Ecuadorian infants, aged 3-23 months, whose mothers were exposed to unspecified OPs during pregnancy through work in the cut-flower industry, had nearly five times higher odds of poor visual acuity, compared to infants whose mothers did not work in the industry (Handal, et al., 2008). We similarly found deficits in visual acuity at 9 months in infants prenatally exposed to chlorpyrifos.

Our previous small pilot study of 9-month-old Chinese infants found that number of pesticides (mixed classes, including OPs) detected in cord blood was positively associated with ABR wave V latencies and CCTs (Sturza, et al., 2016). The association with CCT was additionally modified by iron status, where effect estimates were larger in the low cord ferritin group. The pilot study did not find

any significant associations between number of OP detects and ABR latencies and no individual OPs were examined (due to detection rates <50%) (Sturza, et al., 2016). We similarly did not find any associations between number of OP detects or individual OPs and ABR latencies, and, contrary to these early pilot results, did not see any effect modification by iron status. The number of iron deficient infants with ABR data was small (n=30, 30, and 16, for 6 weeks, 9 months, and 18 months, respectively), which may have limited our power to detect an effect.

Berkowitz and colleagues previously reported inverse associations between maternal urinary metabolites of chlorpyrifos during the third trimester of pregnancy and head circumference at birth, but only after controlling for maternal paraoxonase (PON1) levels (Berkowitz, et al., 2004). We similarly found significant deficits in 9-month head circumference following prenatal chlorpyrifos exposure. Though it is unclear why we observe significant effects on grating VA and head circumference at 9 months only, and not the earlier or later time point. It may be the case that 6 weeks is too early observe significant effects on these outcomes. Assessment of infants at this very young age may be likely to increase the chance of error, especially for the VA measurement. It is unclear why we don't see any effects at the 18-month time point. The smaller sample size at the 18-month time point may have limited our power to detect an effect.

Occupational studies and case studies of OP exposure provide evidence of adverse ocular and auditory effects in adults. Vision loss (Pham, et al., 2016), retinopathy (Pham, et al., 2016), myopia (Dementi, 1994), Saku disease

(Dementi, 1994), and retinal (Dementi, 1994) and macular degeneration (Misra, Nag, Misra, Mehra, & Ray, 1985) have all been reported in rural workers exposed to high levels of OPs. Auditory-related abnormalities such as hearing loss (Hoshino, Pacheco-Ferreira, Taguchi, Tomita, & Miranda Mde, 2008), deficits in auditory temporal processing (Camarinha, Frota, Pacheco-Ferreira, & Lima, 2011), and delays in auditory stimulation classification (Dassanayake, et al., 2008) have also been found in OP-exposed workers.

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

Changes in brain morphology following low-dose early-life chlorpyrifos exposure have also been reported in laboratory rats and human children. Chlorpyrifos in the early postnatal period has been shown to affect both numbers

and types glial cells and neurons in four brain regions associated with cognition, mood, and behavior: the hippocampus, striatum, septal nucleus, and somatosensory cortex in rodents (Roy, Seidler, & Slotkin, 2004; Roy, Sharma, Seidler, & Slotkin, 2005). Postnatal chlorpyrifos has also been associated with glial scarring, a common response to cellular injury (Roy, et al., 2005), while prenatal chlorpryifos was associated with glial cell markers (Garcia, Seidler, Qiao, & Slotkin, 2002). A recent study of 5- to 11-year-old school children similarly found that prenatal chlorpyrifos exposure was associated with enlargements of white matter in brain regions associated with language, cognition, attention, emotion, and inhibitory control: the superior temporal (MST), posterior middle temporal (MT), and inferior postcentral gyri, and the frontal gyrus, gyrus rectus, cuneus, and precuneus along the mesial wall of the right hemisphere (V. A. Rauh, et al., 2012). The authors speculated that the increased white matter may be representative of glial scarring, similar to the effects seen in the rodents (V. A. Rauh, et al., 2012; Roy, et al., 2005). Both the MST and the MT are part of the extrastriate visual cortex, on the dorsal stream pathway from the primary visual cortex (Blumberg & Kreiman, 2010). MT and MST are primarily involved in processing visuospatial information, including the detection of motion, position, and depth perception (Born & Bradley, 2005; Maunsell, 1995). Lesions in the MT have been associated with visual deficits in monkeys, especially with regards to their motion perception (Born & Bradley, 2005). The cuneus is also thought to play a role in the signaling between the primary visual cortex and the extrastriate visual cortices (Vanni, Tanskanen,

Seppa, Uutela, & Hari, 2001). It is unclear whether the MST, MT, or cuneus might also be related to grating acuity in infancy. Given that prenatal chlorpyrifos has been associated with increased white matter or glial scarring in the MST or MT in children, it is possible that this may be one pathway that chlorpyrifos could possibly affect visual function.

Our study has some limitations. OPs are non-persistent with short half-lives. Thus, having measures of exposure only at birth limited our ability to address the temporal variability of OP exposure during pregnancy and infancy. Due to this shortcoming, we were unable to characterize exposure at all sensitive developmental stages (Eskenazi, et al., 2007). Since this was part of a larger study of 96 pesticides and metabolites, our methods were not optimized for OP detection (Silver, Shao, et al., 2016), likely resulting in higher detection limits and great numbers of non-detects, compared to a more targeted approach. OP levels in blood tend to be low anyway, likely also contributing to the high levels of non-detects (Barr, et al., 1999). The many non-detects necessitated the use of crude exposure categories (<LOD/detect or <LOD/medium/high) for nearly all of the OPs examined, thereby limiting the scope of our statistical analyses. Additionally, although our neurodevelopmental testers were highly trained, assessing young infants, as we did here, does augment the chance of error, particularly for grating VA. Furthermore, solely presenting the VA gratings in descending order, as is recommended in the testing manual, may result in habituation, which could possibly confound the estimated VA score. This issue can be addressed by presenting the cards in both descending and ascending

order, however limitations in the attention spans of young infants necessitated a quick completion of the testing, thereby eliminating this as a viable option. Finally, the findings from our relatively small cohort may not be generalizable to infants in other parts of the world, especially considering that all the infants included in this study were carried to term and otherwise healthy. Low birth weight or pre-term infants may be more likely to have delayed development and the effects of prenatal OPs on infant sensory function in these vulnerable populations should be assessed in future work.

Despite its limitations, this study has a number of strengths. It used specific measurements of OP parent compounds in umbilical cord blood to assign prenatal exposure, rather than non-specific metabolites in maternal urine, thus providing direct evidence of fetal exposure (Barr, et al., 1999; Munoz-Quezada, et al., 2013), and providing important information for regulatory considerations. Additionally, OP levels in cord blood may be more likely to reflect the available dose, since the OPs have not yet been eliminated from the infant's body (Needham, Ashley, & Patterson, 1995). Of the previously published studies of prenatal OPs and visual or auditory function, one used a crude "number of pesticide detects" in cord blood to define exposure (Sturza, et al., 2016), while the other used self-reported maternal occupational exposure during pregnancy (Handal, et al., 2008). The current study also examined a large number of OPs (18 detected out of 30 analyzed), many of which have not been previously examined for neurodevelopmental effects in humans. Additionally, we assessed sensory development at three time points (6 weeks, 9 months, and 18 months).

The longitudinal design gives a more comprehensive view of overall sensory development in infancy than previous studies. The tests of sensory function, ABR and grating VA, provided a non-invasive way of measuring auditory and visual function and maturation throughout infancy.

Conclusions

Prenatal exposure to chlopyrifos was significantly associated with deficits in grating visual acuity at 9 months. Chlorpyrifos and phorate were also both associated with deficits in head circumference at 9 months. The clinical significance of these small but statistically significant deficits are unclear, yet warrant further study given that chlorpyrifos and phorate are used worldwide. The proper maturation of the visual and auditory pathways in infancy provides the foundation for later learning processes in childhood. Disruption of this essential neurodevelopmental stage could potentially have detrimental effects on downstream cognition.

Acknowledgments

This study was funded by R01ES021465 from the National Institute of Environmental Health Sciences (NIEHS), P01HD39386 from the National Institute of Child Health and Development (NICHD), and 81273085 from the National Natural Science Foundation of China (NNSFC).

I would also like to acknowledge my collaborators and coauthors on this work: Jie Shao, Lin Xu, Mingyan Li, Minjian Chen, Yankai Xia, Niko Kaciroti, Betsy Lozoff and John Meeker. I would additionally like to thank Twila Tardif for her assistance in interpreting the ABR testing data.

References

Abdollahi, M., & Karami-Mohajeri, S. (2012). A comprehensive review on experimental and clinical findings in intermediate syndrome caused by organophosphate poisoning. *Toxicol Appl Pharmacol, 258*(3), 309-314.

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*(2), 217-223.

Bai, L., Morton, L. C., & Liu, Q. (2013). Climate change and mosquito-borne diseases in China: a review. *Global Health, 9*, 10.

Barr, D. B., Barr, J. R., Driskell, W. J., Hill, R. H., Jr., Ashley, D. L., Needham, L. L., et al. (1999). Strategies for biological monitoring of exposure for contemporary-use pesticides. *Toxicol Ind Health, 15*(1-2), 168-179.

Berkowitz, G. S., Wetmur, J. G., Birman-Deych, E., Obel, J., Lapinski, R. H., Godbold, J. H., et al. (2004). In utero pesticide exposure, maternal paraoxonase activity, and head circumference. *Environ Health Perspect, 112*(3), 388-391.

Blumberg, J., & Kreiman, G. (2010). How cortical neurons help us see: visual recognition in the human brain. *J Clin Invest, 120*(9), 3054-3063.

Born, R. T., & Bradley, D. C. (2005). Structure and function of visual area MT. *Annu Rev Neurosci, 28*, 157-189.

Bouchard, M. F., Chevrier, J., Harley, K. G., Kogut, K., Vedar, M., Calderon, N., et al. (2011). Prenatal exposure to organophosphate pesticides and IQ in 7-year-old children. *Environ Health Perspect, 119*(8), 1189-1195.

Camarinha, C. R., Frota, S. M., Pacheco-Ferreira, H., & Lima, M. A. (2011). [Auditory temporal processing assessment in rural workers exposed to organophosphate pesticides]. *J Soc Bras Fonoaudiol, 23*(2), 102-106.

Chen, C., Qian, Y., Liu, X., Tao, C., Liang, Y., & Li, Y. (2012). Risk assessment of chlorpyrifos on rice and cabbage in China. *Regul Toxicol Pharmacol, 62*(1), 125-130.

Chonchaiya, W., Tardif, T., Mai, X., Xu, L., Li, M., Kaciroti, N., et al. (2013). Developmental trends in auditory processing can provide early predictions of language acquisition in young infants. *Dev Sci, 16*(2), 159-172.

Dassanayake, T., Weerasinghe, V., Dangahadeniya, U., Kularatne, K., Dawson, A., Karalliedde, L., et al. (2008). Long-term event-related potential changes following organophosphorus insecticide poisoning. *Clin Neurophysiol, 119*(1), 144-150.

DeBonis, D. A., & Donohue, C. L. (2008). *Survey of Audiology: Fundamentals for Audiologists and Health Professionals (2nd ed.)*. Boston: Allyn & Bacon.

Dementi, B. (1994). Ocular effects of organophosphates: a historical perspective of Saku disease. *J Appl Toxicol, 14*(2), 119-129.

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

Eddleston, M., Buckley, N. A., Eyer, P., & Dawson, A. H. (2008). Management of acute organophosphorus pesticide poisoning. *Lancet, 371*(9612), 597-607.
Engel, S. M., Wetmur, J., Chen, J., Zhu, C., Barr, D. B., Canfield, R. L., et al. (2011). Prenatal exposure to organophosphates, paraoxonase 1, and cognitive development in childhood. *Environ Health Perspect, 119*(8), 1182-1188.
Eskenazi, B., Marks, A. R., Bradman, A., Harley, K., Barr, D. B., Johnson, C., et al. (2007). Organophosphate pesticide exposure and neurodevelopment in young Mexican-American children. *Environ Health Perspect, 115*(5), 792-798.
Garcia, S. J., Seidler, F. J., Qiao, D., & Slotkin, T. A. (2002). Chlorpyrifos targets developing glia: effects on glial fibrillary acidic protein. *Brain Res Dev Brain Res, 133*(2), 151-161.
Garcia, S. J., Seidler, F. J., & Slotkin, T. A. (2005). Developmental neurotoxicity of chlorpyrifos: targeting glial cells. *Environ Toxicol Pharmacol, 19*(3), 455-461.
Hall, J. I. (2007). *The new handbook of auditory evoked responses*. Boston: Allyn & Bacon.
Handal, A. J., Harlow, S. D., Breilh, J., & Lozoff, B. (2008). Occupational exposure to pesticides during pregnancy and neurobehavioral development of infants and toddlers. *Epidemiology, 19*(6), 851-859.
Hecox, K., & Galambos, R. (1974). Brain stem auditory evoked responses in human infants and adults. *Arch Otolaryngol, 99*(1), 30-33.
Hoshino, A. C., Pacheco-Ferreira, H., Taguchi, C. K., Tomita, S., & Miranda Mde, F. (2008). Ototoxicity study in workers exposed to organophosphate. *Braz J Otorhinolaryngol, 74*(6), 912-918.
Huang, J., Qiao, F., Zhang, L., & Rozelle, S. (2001). *Farm pesticide, rice production, and human health*.
Jiang, Z. D. (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*(1), 49-65.
Kamanyire, R., & Karalliedde, L. (2004). Organophosphate toxicity and occupational exposure. *Occup Med (Lond), 54*(2), 69-75.
Marks, A. R., Harley, K., Bradman, A., Kogut, K., Barr, D. B., Johnson, C., et al. (2010). Organophosphate pesticide exposure and attention in young Mexican-American children: the CHAMACOS study. *Environ Health Perspect, 118*(12), 1768-1774.
Maunsell, J. H. (1995). The brain's visual world: representation of visual targets in cerebral cortex. *Science, 270*(5237), 764-769.
Misra, U. K., Nag, D., Misra, N. K., Mehra, M. K., & Ray, P. K. (1985). Some observations on the macula of pesticide workers. *Hum Toxicol, 4*(2), 135-145.
Munoz-Quezada, M. T., Lucero, B. A., Barr, D. B., Steenland, K., Levy, K., Ryan, P. B., et al. (2013). Neurodevelopmental effects in children associated

with exposure to organophosphate pesticides: a systematic review. *Neurotoxicology, 39*, 158-168.
Needham, L. L., Ashley, D. L., & Patterson, D. G., Jr. (1995). Case studies of the use of biomarkers to assess exposures. *Toxicol Lett, 82-83*, 373-378.
NPIC. (2010). Chlorpyrifos General Fact Sheet. Retrieved June 9, 2016, from www.npic.orst.edu/factsheets/chlorpgen.html
Perez, J. J., Williams, M. K., Weerasekera, G., Smith, K., Whyatt, R. M., Needham, L. L., et al. (2010). Measurement of pyrethroid, organophosphorus, and carbamate insecticides in human plasma using isotope dilution gas chromatography-high resolution mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci, 878*(27), 2554-2562.
Pham, H., Lingao, M. D., Ganesh, A., Capasso, J. E., Keep, R., Sadagopan, K. A., et al. (2016). Organophosphate retinopathy. *Oman J Ophthalmol, 9*(1), 49-51.
Rauh, V., Arunajadai, S., Horton, M., Perera, F., Hoepner, L., Barr, D. B., et al. (2011). Seven-year neurodevelopmental scores and prenatal exposure to chlorpyrifos, a common agricultural pesticide. *Environ Health Perspect, 119*(8), 1196-1201.
Rauh, V. A., Garfinkel, R., Perera, F. P., Andrews, H. F., Hoepner, L., Barr, D. B., et al. (2006). Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. *Pediatrics, 118*(6), e1845-1859.
Rauh, V. A., Perera, F. P., Horton, M. K., Whyatt, R. M., Bansal, R., Hao, X., et al. (2012). Brain anomalies in children exposed prenatally to a common organophosphate pesticide. *Proc Natl Acad Sci U S A, 109*(20), 7871-7876.
Rothenberg, S. J., Poblano, A., & Schnaas, L. (2000). Brainstem auditory evoked response at five years and prenatal and postnatal blood lead. *Neurotoxicol Teratol, 22*(4), 503-510.
Roy, T. S., Seidler, F. J., & Slotkin, T. A. (2004). Morphologic effects of subtoxic neonatal chlorpyrifos exposure in developing rat brain: regionally selective alterations in neurons and glia. *Brain Res Dev Brain Res, 148*(2), 197-206.
Roy, T. S., Sharma, V., Seidler, F. J., & Slotkin, T. A. (2005). Quantitative morphological assessment reveals neuronal and glial deficits in hippocampus after a brief subtoxic exposure to chlorpyrifos in neonatal rats. *Brain Res Dev Brain Res, 155*(1), 71-80.
Shelton, J. F., Geraghty, E. M., Tancredi, D. J., Delwiche, L. D., Schmidt, R. J., Ritz, B., et al. (2014). Neurodevelopmental disorders and prenatal residential proximity to agricultural pesticides: the CHARGE study. *Environ Health Perspect, 122*(10), 1103-1109.
Silver, M. K., Li, X., Liu, Y., Li, M., Mai, X., Kaciroti, N., et al. (2016). Low-level prenatal lead exposure and infant sensory function. *Environ Health, 15*(1), 65.
Silver, M. K., Shao, J., Chen, M., Xia, Y., Lozoff, B., & Meeker, J. D. (2016). Distribution and Predictors of Pesticides in the Umbilical Cord Blood of Chinese Newborns. *Int J Environ Res Public Health, 13*(1).

Slotkin, T. A. (2004). Guidelines for developmental neurotoxicity and their impact on organophosphate pesticides: a personal view from an academic perspective. *Neurotoxicology, 25*(4), 631-640.
Sturza, J., Silver, M. K., Xu, L., Li, M., Mai, X., Xia, Y., et al. (2016). Prenatal exposure to multiple pesticides is associated with auditory brainstem response at 9months in a cohort study of Chinese infants. *Environ Int, 92-93*, 478-485.
Tau, G. Z., & Peterson, B. S. (2010). Normal development of brain circuits. *Neuropsychopharmacology, 35*(1), 147-168.
ThermoScientific. Rapid analysis of pesticides in difficult matrices using GC/MS/MS. Retrieved September 15, 2013, from http://www.analiticaweb.com.br/newsletter/09/51880_RapidAnalysisPesticides.pdf
U.S. CDC. (2016). Parasites- Lice- Head Lice- Treatment. Retrieved October 31, 2016, from www.cdc.gov/parasites/lice/head/treatment.html
U.S. EPA. (2011). Pesticide industry sales and usage: 2006 and 2007 market estimates. Retrieved March 13, 2013, from http://www.panna.org/sites/default/files/EPA%20market_estimates2007.pdf
Vanni, S., Tanskanen, T., Seppa, M., Uutela, K., & Hari, R. (2001). Coinciding early activation of the human primary visual cortex and anteromedial cuneus. *Proc Natl Acad Sci U S A, 98*(5), 2776-2780.
Wang, L., Liang, Y., & Jiang, X. (2008). Analysis of eight organophosphorus pesticide residues in fresh vegetables retailed in agricultural product markets of Nanjing, China. *Bull Environ Contam Toxicol, 81*(4), 377-382.
Wang, S., Wang, Z., Zhang, Y., Wang, J., & Guo, R. (2013). Pesticide residues in market foods in Shaanxi Province of China in 2010. *Food Chem, 138*(2-3), 2016-2025.
Wickerham, E. L., Lozoff, B., Shao, J., Kaciroti, N., Xia, Y., & Meeker, J. D. (2012). Reduced birth weight in relation to pesticide mixtures detected in cord blood of full-term infants. *Environ Int, 47*, 80-85.
Yang, C. C., & Deng, J. F. (2007). Intermediate syndrome following organophosphate insecticide poisoning. *J Chin Med Assoc, 70*(11), 467-472.
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*(2), 125-144.
Zhang, Y., Han, S., Liang, D., Shi, X., Wang, F., Liu, W., et al. (2014). Prenatal exposure to organophosphate pesticides and neurobehavioral development of neonates: a birth cohort study in shenyang, china. *PLoS One, 9*(2), e88491.

Table IV.1. Study sample characteristics

Demographics			**Exposures (cord blood)**		
Variable	**N**	**Mean (SD)**	**Variable**	**N**	**Mean (SD)**
Age (days) at 6 wk testing	196	43.3 (5.1)	# OP detects	200	3.0 (1.6)
Age (days) at 9 m testing	200	282.8 (10.4)	Naled (ng/mL)	200	2.0 (2.8) [GM]
Age (days) at 18 m testing	179	554.7 (10.6)			**N (%)**
Gestational age (weeks)	200	39.6 (1.0)	Methamidophos (ng/mL)	200	
Birth weight (kg)	200	3.4 (0.4)	High (>18.2)		64 (32.0)
6 wk head circ. (cm)	199	38.0 (1.2)	Medium (1.5-18.2)		65 (32.5)
9 m head circ. (cm)	199	45.1 (1.5)	<LOD (<1.5)		71 (35.5)
18 m head circ. (cm)	186	47.4 (1.4)	Trichlorfon (ng/mL)	200	
		N (%)	High (>1.7)		50 (25.0)
Sex	200		Medium (0.4-1.7)		52 (26.0)
Male		107 (53.5)	<LOD (<0.4)		98 (49.0)
Female		93 (46.5)	Chlorpyrifos (ng/mL)	200	
Maternal occupation	186		Detect (≥0.4)		72 (36.0)
Housewife		75 (40.3)	ND (<0.4)		128 (64.0)
Other		111 (59.7)	Phorate (ng/mL)	200	
Maternal education	186		Detect (≥1.8)		36 (18.0)
College		62 (33.3)	ND (<1.8)		164 (82.0)
High/secondary school		53 (28.5)	Serum ferritin (μg/L)	199	
Middle school or less		71 (38.2)	Normal (75-370)		160 (80.4)
Family income (Yuan/year)	183		Low (≤75)		39 (19.6)
≥ 100,000		52 (28.4)			
50,000-999,999		58 (31.7)			
30,000-49,999		35 (19.1)			
<30,000		38 (20.1)			

GM- denotes geometric mean

Table IV.2. Adjusted [a] longitudinal change/difference in VA score by OP exposure

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

[a] Models adjusted for sex, age at testing, and cord ferritin
[b] Estimated change in VA score per 1 unit increase in OP
[c] Difference in mean VA score
High/Medium/ND cut-offs (ng/mL): methamidophos >18.2/1.5-18.2/ND; trichlorfon >1.7/ 0.4-1.7/ND; chlorpyrifos ≥0.04/ND; phorate ≥1.8/ND
Abbreviations: VA, grating visual acuity
*p<0.05

Table IV.3. Adjusted [a] longitudinal change/difference in ABR latencies by OP exposure

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

[a] Models adjusted for sex, age at testing, and cord ferritin
[b] Estimated change in ABR latency per 1 unit increase in OP
[c] Difference in mean ABR latency
High/Medium/ND cut-offs (ng/mL): methamidophos >18.2/1.5-18.2/ND; trichlorfon >1.7/ 0.4-1.7/ND; chlorpyrifos ≥0.04/ND; phorate ≥1.8/ND
Abbreviations: ABR, auditory brainstem response; CCT, central conduction time
†p<0.10, *p<0.05

Table IV.3, continued

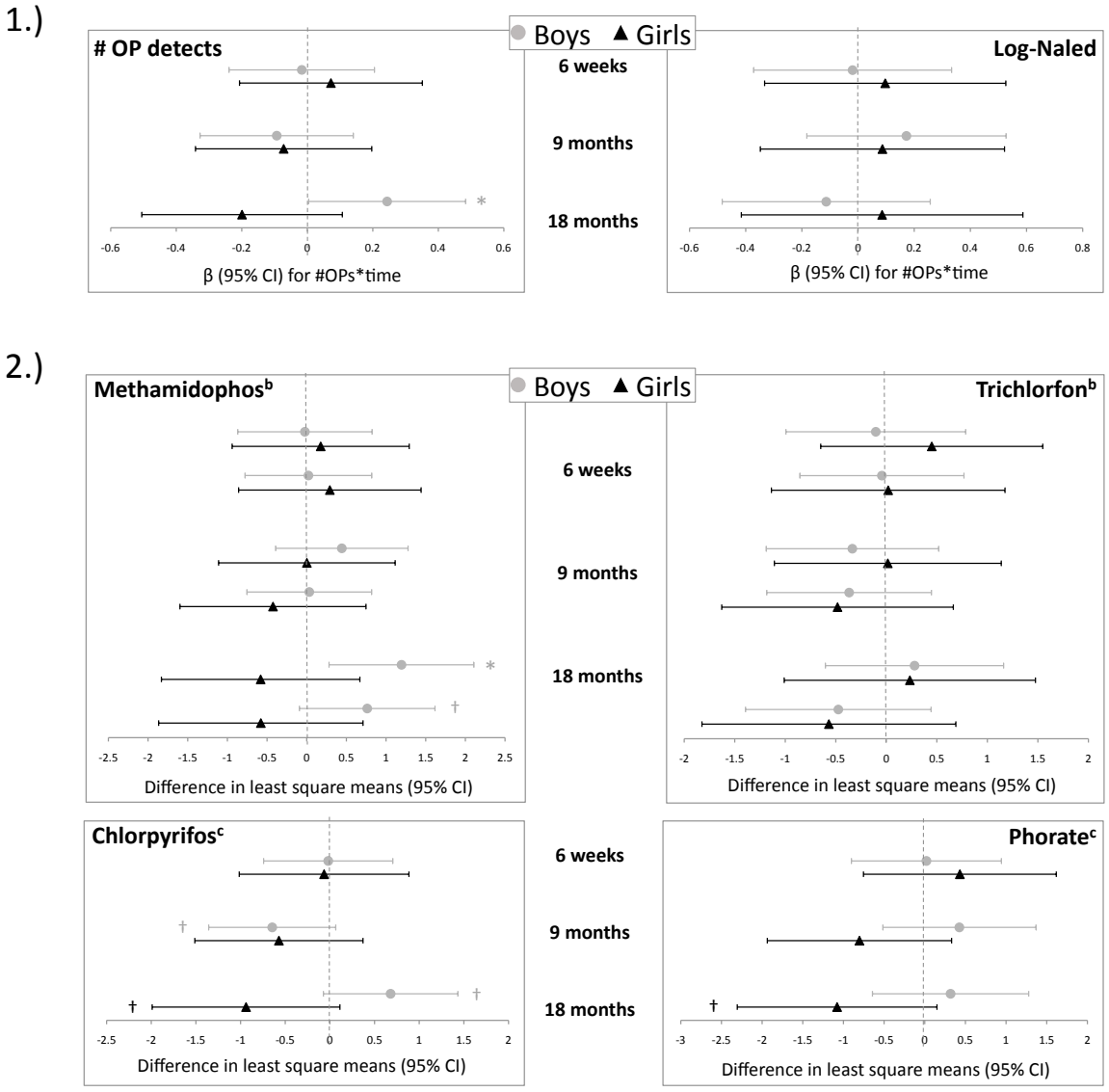
	ABR outcome					
	6 weeks		9 months		18 months	
OP insecticide	IPI III-V (n=181)	IPI-I-III (n=180)	IPI III-V (n=147)	IPI-I-III (n=94)	IPI III-V (n=94)	IPI-I-III (n=94)
Continuous	**β (95% CI) for OP [b]**					
# OP Detects	0.00 (-0.01, 0.02)	0.01 (-0.01, 0.02)	-0.01 (-0.02, 0.01)	0.00 (-0.01, 0.02)	0.00 (-0.02, 0.02)	0.00 (-0.02, 0.02)
Log-Naled	0.01 (-0.02, 0.03)	0.01 (-0.02, 0.03)	0.00 (-0.02, 0.03)	0.01 (-0.01, 0.04)	0.00 (-0.03, 0.03)	0.00 (-0.02, 0.03)
3-level (High/Med./ND)	**Difference in least square means (95% CI) [c]**					
Methamidophos (High vs ND)	0.02 (-0.04, 0.08)	0.05 (-0.01, 0.11) †	0.00 (-0.06, 0.07)	0.06 (0.00, 0.12) †	0.04 (-0.04, 0.11)	0.02 (-0.04, 0.09)
Methamidophos (Med. vs ND)	0.02 (-0.08, 0.04)	0.01 (-0.05, 0.06)	-0.01 (-0.08, 0.05)	-0.01 (-0.07, 0.05)	0.01 (-0.07, 0.09)	0.00 (-0.07, 0.07)
Trichlorfon (High vs ND)	0.05 (-0.01, 0.11)	-0.03 (-0.08, 0.03)	0.00 (-0.06, 0.07)	-0.01 (-0.07, 0.05)	0.00 (-0.08, 0.07)	0.01 (-0.06, 0.08)
Trichlorfon (Med. vs ND)	0.04 (-0.02, 0.11)	-0.05 (-0.11, 0.01) †	0.00 (-0.07, 0.07)	-0.04 (-0.10, 0.02)	0.02 (-0.05, 0.10)	-0.04 (-0.11, 0.03)
2-level (Detect/ND)	**Difference in least square means (95% CI) [c]**					
Chlorpyrifos (Detect vs ND)	0.00 (-0.05, 0.05)	-0.01 (-0.06, 0.04)	-0.01 (-0.06, 0.05)	0.00 (-0.05, 0.05)	-0.01 (-0.07, 0.06)	-0.03 (-0.09, 0.03)
Phorate (Detect vs ND)	0.04 (-0.03, 0.10)	0.00 (-0.06, 0.06)	-0.04 (-0.11, 0.03)	0.02 (-0.05, 0.08)	0.00 (-0.08, 0.08)	-0.01 (-0.08, 0.06)

[a] Models adjusted for sex, age at testing, and cord ferritin
[b] Estimated change in ABR latency per 1 unit increase in OP
[c] Difference in mean ABR latency
High/Medium/ND cut-offs (ng/mL): methamidophos >18.2/1.5-18.2/ND; trichlorfon >1.7/ 0.4-1.7/ND; chlorpyrifos ≥0.04/ND; phorate ≥1.8/ND
Abbreviations: ABR, auditory brainstem response; IPI, inter-peak interval
†p<0.10, *p<0.05

Table IV.4. Comparison of grating VA scores by infant sex

	Overall		Boys		Girls		Test statistic
Time point	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	T (p)
6 weeks	177	1.17 (0.55)	103	1.07 (0.37)	93	1.28 (0.68)	-2.63 (0.01)
9 months	200	7.65 (2.33)	107	7.77 (2.18)	93	7.52 (2.51)	0.74 (0.46)
18 months	158	9.48 (2.49)	95	9.49 (2.14)	84	9.42 (3.00)	0.17 (0.86)

Figure IV.1. Sex-stratified change/difference (95%) in grating VA scores by OP exposure [a]



1.) Estimated change in grating VA score per 1 unit increase in OP exposure
2.) Difference in mean grating VA score by category of OP exposure

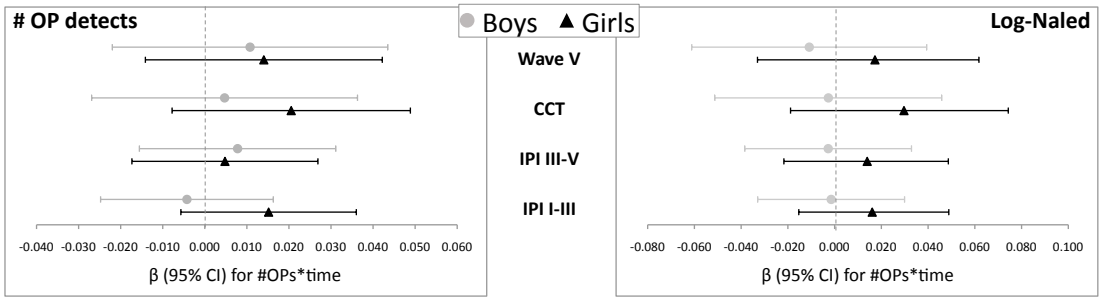
[a] Models adjusted for age at testing and cord ferritin
[b] Categories of OP exposure: high versus ND and medium versus ND
[c] Categories of OP exposure: exposed versus ND
High/Medium/ND cut-offs (ng/mL): methamidophos >18.2/1.5-18.2/ND; trichlorfon >1.7/ 0.4-1.7/ND; chlorpyrifos ≥0.04/ND; phorate ≥1.8/ND
†p<0.10, *p<0.05

Table IV.5. Comparison of key ABR latency variables by infant sex

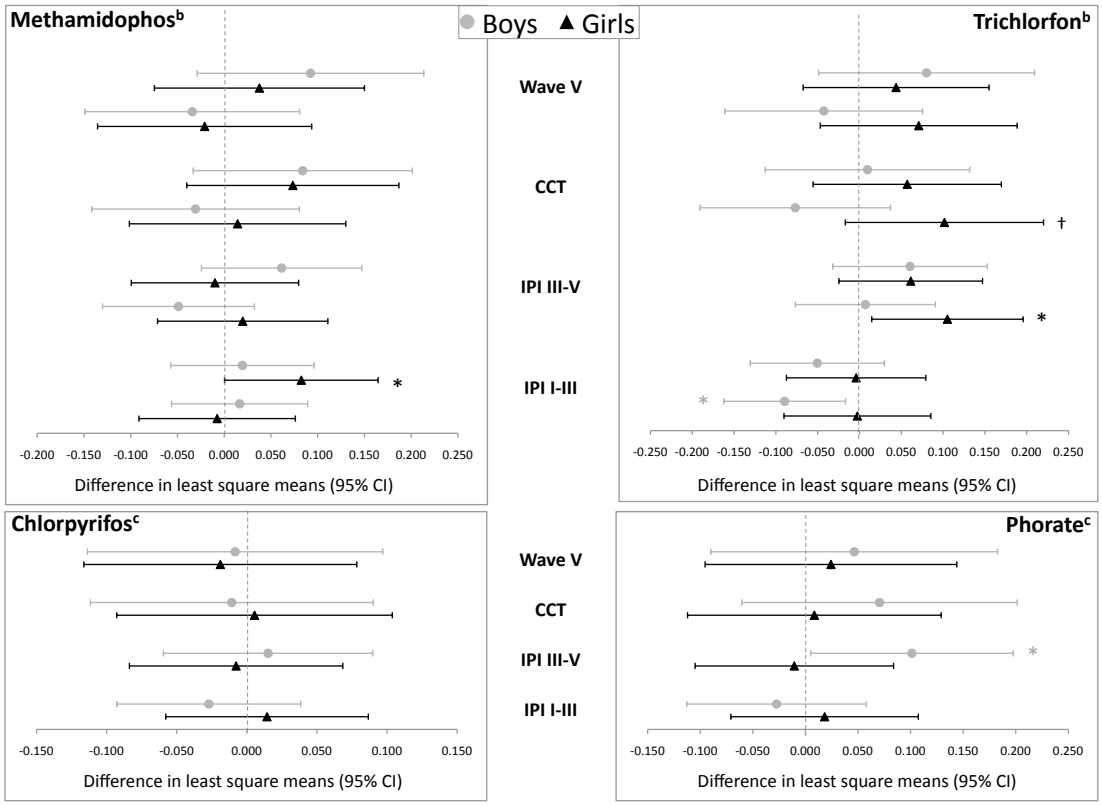
Time point	Overall		Boys		Girls		Test statistic
Wave V	**n**	**Mean (SD)**	**n**	**Mean (SD)**	**n**	**Mean (SD)**	**T (p)**
6 weeks	183	6.45 (0.26)	95	6.55 (0.27)	88	6.34 (0.22)	5.48 (<0.0001)
9 months	176	5.90 (0.26)	91	5.98 (0.26)	85	5.81 (0.23)	4.61 (<0.0001)
18 months	139	5.71 (0.23)	73	5.80 (0.20)	66	5.61 (0.22)	5.39 (<0.0001)
CCT							
6 weeks	182	4.94 (0.27)	94	5.05 (0.26)	88	4.83 (0.23)	6.12 (<0.0001)
9 months	153	4.40 (0.25)	77	4.49 (0.24)	76	4.31 (0.23)	4.77 (<0.0001)
18 months	106	4.20 (0.21)	55	4.27 (0.19)	51	4.13 (0.21)	3.53 (0.001)

Figure IV.2. Sex-stratified change/difference (95%) in 6-week ABR scores by OP exposure [a]

1.)



2.)



1.) Estimated change in ABR latency per 1 unit increase in OP exposure
2.) Difference in mean ABR latency by category of OP exposure

[a] Models adjusted for age at testing and cord ferritin
[b] Categories of OP exposure: high versus ND and medium versus ND
[c] Categories of OP exposure: exposed versus ND
High/Medium/ND cut-offs (ng/mL): methamidophos >18.2/1.5-18.2/ND; trichlorfon >1.7/ 0.4-1.7/ND; chlorpyrifos ≥0.04/ND; phorate ≥1.8/ND
†p<0.10, *p<0.05, **p<0.01

Table IV.6. Adjusted [a] longitudinal change/difference in head circumference by OP exposure

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

[a] Models adjusted for sex, age, and cord ferritin
[b] Estimated change in head circumference per 1 unit increase in OP
[c] Difference in mean head circumference
High/Medium/ND cut-offs (ng/mL): methamidophos >18.2/1.5-18.2/ND; trichlorfon >1.7/ 0.4-1.7/ND; chlorpyrifos ≥0.04/ND; phorate ≥1.8/ND
†p<0.10, *p<0.05

Chapter IV Appendix

Table IV.A1. Comparison of OP exposures for infants with and without sensory data

	Grating VA								
	6-week data?			9-month data?			18-month data?		
OP Insecticide	**Yes (n=196)**	**No (n=42)**	**Test**	**Yes (n=200)**	**No (n=38)**	**Test**	**Yes (n=179)**	**No (n=59)**	**Test**
Continuous	**Mean (SD)**	**Mean (SD)**	**T (p)**	**Mean (SD)**	**Mean (SD)**	**T (p)**	**Mean (SD)**	**Mean (SD)**	**T (p)**
# OP detects	3.0 (1.6)	3.0 (1.4)	-0.0 (1.0)	3.0 (1.6)	2.9 (1.5)	-0.3 (0.8)	3.1 (1.6)	2.7 (1.5)	-1.7 (0.1)
Log-naled	0.8 (1.0)	0.6 (0.9)	-0.9 (0.4)	0.8 (1.0)	0.6 (0.9)	-0.8 (0.4)	0.7 (1.0)	0.7 (1.0)	-0.3 (0.8)
Categorical	**N (%)**	**N (%)**	**X^2 (p)**	**N (%)**	**N (%)**	**X^2 (p)**	**N (%)**	**N (%)**	**X^2 (p)**
Methamidophos			1.2 (0.6)			0.1 (1.0)			0.5 (0.8)
High	61 (31.1)	16 (38.1)		64 (32.0)	13 (34.2)		58 (32.4)	19 (32.2)	
Medium	63 (32.1)	14 (33.3)		65 (32.5)	12 (31.6)		56 (31.3)	21 (35.6)	
<LOD	72 (36.7)	12 (28.6)		71 (35.5)	13 (34.2)		65 (36.3)	19 (32.2)	
Trichlorfon			0.3 (0.9)			0.3 (0.9)			2.3 (0.3)
High	49 (25.0)	12 (28.6)		50 (25.0)	11 (29.0)		50 (27.9)	11 (18.6)	
Medium	51 (26.0)	10 (23.8)		52 (26.0)	9 (23.7)		46 (25.7)	15 (25.4)	
<LOD	96 (49.0)	20 (47.6)		98 (49.0)	18 (47.4)		83 (46.4)	33 (55.9)	
Chlorpyrifos			1.7 (0.2)			0.2 (0.7)			3.0 (0.1)
≥LOD	68 (34.7)	19 (45.2)		72 (36.0)	15 (39.5)		71 (39.7)	16 (27.1)	
<LOD	128 (65.3)	23 (54.8)		128 (64.0)	23 (60.5)		108 (60.3)	43 (72.9)	
Phorate			0.3 (0.6)			0.5 (0.5)			2.7 (0.1)
≥LOD	35 (17.9)	6 (14.3)		36 (18.0)	5 (13.2)		35 (19.6)	6 (10.2)	
<LOD	161 (82.1)	36 (85.7)		164 (82.0)	33 (86.8)		144 (80.5)	53 (89.8)	

Table IV.A1, continued

	ABR Wave V								
	6 week data?			9 month data?			18 month data?		
OP Insecticide	**Yes (n=183)**	**No (n=55)**	**Test**	**Yes (n=176)**	**No (n=62)**	**Test**	**Yes (n=139)**	**No (n=99)**	**Test**
Continuous	**Mean (SD)**	**Mean (SD)**	**T (p)**	**Mean (SD)**	**Mean (SD)**	**T (p)**	**Mean (SD)**	**Mean (SD)**	**T (p)**
# OP detects	2.9 (1.6)	3.2 (1.5)	1.0 (0.3)	3.0 (1.6)	2.9 (1.6)	-0.4 (0.7)	3.1 (1.6)	2.9 (1.6)	-0.9 (0.4)
Log-naled	0.8 (1.0)	0.6 (0.9)	-1.5 (0.1)	0.7 (1.0)	0.7 (1.0)	-0.3 (0.7)	0.7 (1.0)	0.8 (1.0)	0.7 (0.5)
Categorical	**N (%)**	**N (%)**	**X^2 (p)**	**N (%)**	**N (%)**	**X^2 (p)**	**N (%)**	**N (%)**	**X^2 (p)**
Methamidophos			0.7 (0.7)			1.0 (0.6)			4.0 (0.1)
High	57 (31.2)	20 (36.4)		58 (33.0)	19 (30.7)		47 (33.8)	30 (30.3)	
Medium	59 (32.2)	18 (32.7)		59 (33.5)	18 (29.0)		38 (27.3)	39 (39.4)	
<LOD	67 (36.6)	17 (30.9)		59 (33.5)	25 (40.3)		54 (38.9)	30 (30.3)	
Trichlorfon			2.1 (0.3)			0.7 (0.7)			1.7 (0.4)
High	43 (23.5)	18 (32.7)		47 (26.7)	14 (22.6)		39 (28.1)	22 (22.2)	
Medium	47 (25.7)	14 (25.5)		43 (24.4)	18 (29.0)		37 (26.6)	24 (24.2)	
<LOD	93 (50.8)	23 (41.8)		86 (48.9)	30 (48.4)		63 (45.3)	53 (53.5)	
Chlorpyrifos			2.4 (0.1)			0.0 (0.9)			0.4 (0.6)
≥LOD	62 (33.9)	25 (45.5)		64 (36.4)	23 (37.1)		53 (38.1)	34 (34.3)	
<LOD	121 (33.9)	30 (54.6)		112 (63.6)	39 (62.9)		86 (61.9)	65 (65.7)	
Phorate			0.0 (0.8)			0.3 (0.6)			1.1 (0.3)
≥LOD	31 (16.9)	10 (18.2)		29 (16.5)	12 (19.4)		27 (19.4)	14 (14.1)	
<LOD	152 (83.1)	45 (81.8)		147 (83.5)	50 (80.7)		112 (80.6)	85 (85.9)	

Table IV.A1, continued

	ABR CCT								
	6 week data?			9 month data?			18 month data?		
OP Insecticide	**Yes (n=182)**	**No (n=56)**	**Test**	**Yes (n=154)**	**No (n=84)**	**Test**	**Yes (n=106)**	**No (n=132)**	**Test**
Continuous	**Mean (SD)**	**Mean (SD)**	**T (p)**	**Mean (SD)**	**Mean (SD)**	**T (p)**	**Mean (SD)**	**Mean (SD)**	**T (p)**
# OP detects	2.9 (1.6)	3.1 (1.5)	0.9 (0.4)	3.0 (1.6)	3.0 (1.6)	0.2 (0.8)	3.1 (1.7)	2.9 (1.5)	-0.9 (0.4)
Log-naled	0.8 (1.0)	0.6 (0.9)	-1.4 (0.2)	0.8 (1.0)	0.7 (1.1)	-0.6 (0.6)	0.7 (1.0)	0.8 (1.0)	0.3 (0.7)
Categorical	**N (%)**	**N (%)**	**X^2 (p)**	**N (%)**	**N (%)**	**X^2 (p)**	**N (%)**	**N (%)**	**X^2 (p)**
Methamidophos			0.8 (0.7)			0.1 (0.9)			8.7 (0.01)
High	57 (31.3)	20 (35.7)		49 (31.8)	28 (33.3)		37 (34.9)	40 (30.3)	
Medium	58 (31.9)	19 (33.9)		51 (33.1)	26 (31.0)		24 (22.6)	53 (40.2)	
<LOD	67 (36.8)	17 (30.4)		54 (35.1)	30 (35.7)		45 (42.5)	39 (29.6)	
Trichlorfon			1.7 (0.4)			0.3 (0.9)			3.0 (0.2)
High	43 (23.6)	18 (32.1)		41 (26.6)	20 (23.8)		30 (28.3)	31 (23.5)	
Medium	47 (25.8)	14 (25.0)		38 (24.7)	23 (27.4)		31 (29.3)	30 (22.7)	
<LOD	92 (50.6)	24 (42.9)		75 (48.7)	41 (48.8)		45 (42.5)	71 (53.8)	
Chlorpyrifos			2.1 (0.2)			1.5 (0.2)			1.3 (0.2)
≥LOD	62 (34.1)	25 (44.6)		52 (33.8)	35 (41.7)		43 (40.6)	44 (33.3)	
<LOD	120 (65.9)	31 (55.4)		102 (66.2)	49 (58.3)		63 (59.4)	88 (66.7)	
Phorate			0.0 (0.9)			0.8 (0.4)			0.9 (0.3)
≥LOD	31 (17.1)	10 (17.9)		24 (15.6)	17 (20.2)		21 (19.8)	20 (15.2)	
<LOD	151 (83.0)	46 (82.1)		130 (84.4)	67 (79.8)		85 (80.2)	112 (84.9)	

Table IV.A2 Sex-stratified change/difference in ABR latencies at 9 months and 18 months by OP exposure [a]

	BOYS at 9 months			
OP insecticide	**Wave V (n=85)**	**CCT (n=74)**	**IPI III-V (n=74)**	**IPI-I-III (n=75)**
Continuous	**β (95% CI) for OP [b]**			
# OP Detects	-0.02	-0.01	-0.01	0.00
	(-0.06, 0.01)	(-0.05, 0.02)	(-0.04, 0.01)	(-0.02, 0.02)
Log-Naled	-0.00	0.01	0.01	0.00
	(-0.05, 0.05)	(-0.04, 0.06)	(-0.03, 0.05)	(-0.03, 0.04)
3-level (High/Med./ND)	**Difference in least square means (95% CI) [c]**			
Methamidophos	-0.01	0.05	0.01	0.03
(High vs ND)	(-0.14, 0.11)	(-0.08, 0.17)	(-0.08, 0.11)	(-0.05, 0.11)
Methamidophos	0.04	0.00	-0.03	0.03
(Med. vs ND)	(-0.08, 0.16)	(-0.11, 0.12)	(-0.11, 0.06)	(-0.04, 0.11)
Trichlorfon	-0.02	-0.01	-0.01	0.00
(High vs ND)	(-0.11, 0.14)	(-0.13, 0.11)	(-0.10, 0.09)	(-0.07, 0.08)
Trichlorfon	-0.10	-0.14 *	-0.07	-0.05
(Med. vs ND)	(-0.21, 0.04)	(-0.26, -0.02)	(-0.17, 0.02)	(-0.14, 0.03)
2-level (Detect/ND)	**Difference in least square means (95% CI) [c]**			
Chlorpyrifos	-0.07	-0.06	-0.03	-0.02
(Detect vs ND)	(-0.18, 0.03)	(-0.17, 0.04)	(-0.12, 0.05)	(-0.10, 0.04)
Phorate	-0.07	-0.03	-0.01	-0.01
(Detect vs ND)	(-0.21, 0.07)	(-0.16, 0.11)	(-0.12, 0.09)	(-0.11, 0.08)
	GIRLS at 9 months			
OP insecticide	**Wave V (n=80)**	**CCT (n=72)**	**IPI III-V (n=73)**	**IPI-I-III (n=73)**
Continuous	**β (95% CI) for OP [b]**			
# OP Detects	-0.01	-0.00	-0.00	0.00
	(-0.04, 0.02)	(-0.03, 0.03)	(-0.03, 0.02)	(-0.02, 0.02)
Log-Naled	-0.00	0.01	-0.00	0.02
	(-0.05, 0.04)	(-0.03, 0.06)	(-0.04, 0.04)	(-0.02, 0.05)
3-level (High/Med./ND)	**Difference in least square means (95% CI) [c]**			
Methamidophos	0.03	0.08	0.00	0.08 †
(High vs ND)	(-0.09, 0.14)	(-0.04, 0.20)	(-0.09, 0.10)	(-0.01, 0.16)
Methamidophos	-0.09	-0.05	0.00	-0.06
(Med. vs ND)	(-0.21, 0.03)	(-0.18, 0.0.07)	(-0.10, 0.10)	(-0.15, 0.03)
Trichlorfon	-0.04	-0.02	0.02	-0.04
(High vs ND)	(-0.16, 0.07)	(-0.14, 0.10)	(-0.07, 0.12)	(-0.13, 0.05)
Trichlorfon	0.03	0.06	0.09 †	-0.03
(Med. vs ND)	(-0.09, 0.16)	(-0.07, 0.18)	(-0.01, 0.18)	(-0.12, 0.06)
2-level (Detect/ND)	**Difference in least square means (95% CI) [c]**			
Chlorpyrifos	0.02	0.03	0.01	0.02
(Detect vs ND)	(-0.08, 0.12)	(-0.08, 0.13)	(-0.07, 0.10)	(-0.06, 0.09)
Phorate	-0.02	0.00	-0.05	0.05
(Detect vs ND)	(-0.15, 0.10)	(-0.13, 0.13)	(-0.16, 0.05)	(-0.05, 0.15)

[a] Models adjusted for age at testing, and cord ferritin
[b] Estimated change in 9-month ABR latency per 1 unit increase in OP
[c] Difference in mean 9-month ABR latency
†p<0.10; *p<0.05
High/Medium/ND cut-offs (ng/mL): methamidophos >18.2/1.5-18.2/ND; trichlorfon >1.7/ 0.4-1.7/ND; chlorpyrifos ≥0.04/ND; phorate ≥1.8/ND
Abbreviations: ABR, auditory brainstem response; CCT, central conduction time; IPI, inter-peak interval

Table IV.A2, continued

	BOYS at 18 months			
OP insecticide	**Wave V (n=68)**	**CCT (n=50)**	**IPI III-V (n=50)**	**IPI-I-III (n=50)**
Continuous	**β (95% CI) for OP [b]**			
# OP Detects	-0.00	-0.01	-0.00	-0.00
	(-0.04, 0.03)	(-0.04, 0.03)	(-0.03, 0.03)	(-0.03, 0.02)
Log-Naled	0.00	0.00	0.01	-0.00
	(-0.05, 0.06)	(-0.06, 0.06)	(-0.04, 0.05)	(-0.04, 0.04)
3-level (High/Med./ND)	**Difference in least square means (95% CI) [c]**			
Methamidophos	-0.00	0.03	0.05	-0.01
(High vs ND)	(-0.13, 0.13)	(-0.10, 0.16)	(-0.06, 0.15)	(-0.10, 0.08)
Methamidophos	-0.00	-0.00	-0.01	0.02
(Med. vs ND)	(-0.12, 0.12)	(-0.13, 0.13)	(-0.11, 0.09)	(-0.07, 0.11)
Trichlorfon	-0.01	-0.01	-0.02	-0.01
(High vs ND)	(-0.14, 0.12)	(-0.14, 0.12)	(-0.12, 0.09)	(-0.10, 0.08)
Trichlorfon	-0.02	-0.09	-0.03	-0.06
(Med. vs ND)	(-0.15, 0.12)	(-0.22, 0.04)	(-0.13, 0.07)	(-0.15, 0.03)
2-level (Detect/ND)	**Difference in least square means (95% CI) [c]**			
Chlorpyrifos	-0.01	-0.04	0.01	-0.05
(Detect vs ND)	(-0.12, 0.11)	(-0.15, 0.07)	(-0.08, 0.09)	(-0.13, 0.03)
Phorate	-0.03	-0.01	0.02	-0.03
(Detect vs ND)	(-0.17, 0.11)	(-0.15, 0.13)	(-0.09, 012)	(-0.13, 0.06)
	GIRLS at 18 months			
OP insecticide	**Wave V (n=58)**	**CCT (n=44)**	**IPI III-V (n=44)**	**IPI-I-III (n=44)**
Continuous	**β (95% CI) for OP [b]**			
# OP Detects	0.00	0.00	-0.00	0.00
	(-0.03, 0.04)	(-0.03, 0.04)	(-0.03, 0.03)	(-0.02, 0.03)
Log-Naled	0.00	-0.00	-0.01	0.01
	(-0.05, 0.05)	(-0.05, 0.05)	(-0.05, 0.04)	(-0.03, 0.05)
3-level (High/Med./ND)	**Difference in least square means (95% CI) [c]**			
Methamidophos	0.06	0.09	0.05	0.04
(High vs ND)	(-0.07, 0.18)	(-0.05, 0.22)	(-0.06, 0.16)	(-0.06, 0.14)
Methamidophos	-0.06	0.02	0.02	-0.01
(Med. vs ND)	(-0.20, 0.08)	(-0.13, 0.17)	(-0.11, 0.15)	(-0.13, 0.10)
Trichlorfon	-0.00	0.06	0.02	0.03
(High vs ND)	(-0.13, 0.13)	(-0.08, 0.20)	(-0.09, 0.14)	(-0.08, 0.14)
Trichlorfon	0.06	0.08	0.10	-0.02
(Med. vs ND)	(-0.07, 0.20)	(-0.06, 0.22)	(-0.02, 0.21)	(-0.13, 0.09)
2-level (Detect/ND)	**Difference in least square means (95% CI) [c]**			
Chlorpyrifos	-0.00	-0.04	-0.02	-0.01
(Detect vs ND)	(-0.12, 0.11)	(-0.15, 0.08)	(-0.11, 0.08)	(-0.10, 0.08)
Phorate	0.08	-0.01	-0.01	0.01
(Detect vs ND)	(-0.05, 0.22)	(-0.15, 0.14)	(-0.13, 0.11)	(-0.10, 0.12)

[a] Models adjusted for age at testing, and cord ferritin
[b] Estimated change in 18-month ABR latency per 1 unit increase in OP
[c] Difference in mean 18-month ABR latency
High/Medium/ND cut-offs (ng/mL): methamidophos >18.2/1.5-18.2/ND; trichlorfon >1.7/ 0.4-1.7/ND; chlorpyrifos ≥0.04/ND; phorate ≥1.8/ND
Abbreviations: ABR, auditory brainstem response; CCT, central conduction time; IPI, inter-peak interval

Table IV.A3. Cord iron-stratified change/difference in ABR latencies at by OP exposure [a]

	Iron sufficient			
	6 weeks			
OP insecticide	**Wave V (n=151)**	**CCT (n=150)**	**IPI III-V (n=151)**	**IPI-I-III (n=150)**
Continuous	**β (95% CI) for OP [b]**			
# OP Detects	0.02 (-0.00, 0.04)	0.02 (-0.00, 0.04)	0.01 (-0.01, 0.02)	0.01 (-0.00, 0.03)
Log-Naled	0.01 (-0.02, 0.05)	0.02 (-0.01, 0.06)	0.01 (-0.01, 0.03)	0.02 (-0.01, 0.04)
3-level (High/Med./ND)	**Difference in least square means (95% CI) [c]**			
Methamidophos (High vs ND)	0.08 † (-0.01, 0.17)	0.09 † (0.00, 0.17)	0.02 (-0.04, 0.09)	0.06 † (0.00, 0.12)
Methamidophos (Med. vs ND)	-0.03 (-0.12, 0.06)	-0.02 (-0.11, 0.06)	-0.03 (-0.10, 0.04)	0.00 (-0.06, 0.06)
Trichlorfon (High vs ND)	0.08 † (-0.01, 0.17)	0.06 (-0.03, 0.14)	0.07 † (0.00, 0.13)	-0.01 (-0.07, 0.05)
Trichlorfon (Med. vs ND)	0.03 (-0.06, 0.12)	0.03 (0.06, 0.11)	0.05 (-0.01, 0.12)	-0.03 (-0.09, 0.03)
2-level (Detect/ND)	**Difference in least square means (95% CI) [c]**			
Chlorpyrifos (Detect vs ND)	0.02 (-0.05, 0.09)	0.01 (-0.06, 0.09)	0.01 (-0.05, 0.07)	0.00 (-0.05, 0.06)
Phorate (Detect vs ND)	0.05 (-0.3, 0.11)	0.06 (-0.03, 015)	0.06 (-0.01, 0.13)	0.01 (-0.06, 0.07)
	Iron deficient			
	6 weeks			
OP insecticide	**Wave V (n=30)**	**CCT (n=30)**	**IPI III-V (n=30)**	**IPI-I-III (n=30)**
Continuous	**β (95% CI) for OP [b]**			
# OP Detects	-0.04 (-0.10, 0.02)	-0.04 (-0.10, 0.03)	-0.01 (-0.05, 0.04)	-0.03 (-0.07, 0.01)
Log-Naled	-0.07 (-0.17, 0.02)	-0.06 (-0.16, 0.05)	-0.01 (-0.08, 0.06)	-0.05 (-0.11, 0.01)
3-level (High/Med./ND)	**Difference in least square means (95% CI) [c]**			
Methamidophos (High vs ND)	-0.12 (-0.37, 0.13)	-0.07 (-0.33, 0.19)	-0.02 (-0.20, 0.17)	-0.06 (-0.22, 0.10)
Methamidophos (Med. vs ND)	-0.03 (-0.24, 0.19)	0.04 (-0.19, 0.27)	0.04 (-0.11, 0.19)	-0.01 (-0.14, 0.13)
Trichlorfon (High vs ND)	-0.14 (-0.38, 0.11)	-0.14 (-0.41, 0.12)	-0.02 (-0.20, 0.17)	-0.12 (-0.28, 0.03)
Trichlorfon (Med. vs ND)	-0.14 (-0.39, 0.12)	-0.20 (-0.48, 0.07)	-0.01 (-0.20, 0.18)	-0.19 * (-0.35, -0.03)
2-level (Detect/ND)	**Difference in least square means (95% CI) [c]**			
Chlorpyrifos (Detect vs ND)	-0.11 (-0.30, 0.07)	-0.08 (-0.28, 0.12)	-0.03 (-0.16, 0.10)	-0.05 (-0.17, 0.07)
Phorate (Detect vs ND)	-0.13 (-0.42, 0.17)	-0.14 (-0.45, 0.16)	-0.07 (-0.28, 0.14)	-0.07 (-0.26, 0.12)

[a] Models adjusted for sex and age at testing
[b] Estimated change in ABR latency per 1 unit increase in OP
[c] Difference in mean ABR latency
High/Medium/ND cut-offs (ng/mL): methamidophos >18.2/1.5-18.2/ND; trichlorfon >1.7/ 0.4-1.7/ND; chlorpyrifos ≥0.04/ND; phorate ≥1.8/ND
Abbreviations: ABR, auditory brainstem response; CCT, central conduction time; IPI, inter-peak interval
†p<0.10, *p<0.05

Table IV.A3, continued

	Iron sufficient			
	9 months			
OP insecticide	**Wave V (n=135)**	**CCT (n=118)**	**IPI III-V (n=118)**	**IPI-I-III (n=119)**
Continuous	**β (95% CI) for OP [b]**			
# OP Detects	-0.02 (-0.04, 0.01)	-0.00 (-0.03, 0.02)	-0.01 (-0.03, 0.01)	0.00 (-0.01, 0.02)
Log-Naled	-0.00 (-0.04, 0.04)	0.01 (-0.02, 0.05)	0.00 (-0.03, 0.03)	0.01 (-0.01, 0.04)
3-level (High/Med./ND)	**Difference in least square means (95% CI) [c]**			
Methamidophos (High vs ND)	-0.00 (-0.09, 0.09)	0.06 (-0.03, 0.15)	0.01 (-0.06, 0.08)	0.05 (-0.02, 0.12)
Methamidophos (Med. vs ND)	-0.03 (-0.12, 0.07)	-0.04 (-0.13, 0.05)	-0.03 (-0.10, 0.05)	-0.01 (-0.08, 0.05)
Trichlorfon (High vs ND)	-0.03 (-0.12, 0.06)	-0.01 (-0.10, 0.08)	0.00 (-0.07, 0.08)	-0.01 (-0.07, 0.06)
Trichlorfon (Med. vs ND)	-0.03 (-0.12, 0.06)	-0.04 (-0.13, 0.05)	0.01 (-0.06, 0.08)	-0.05 (-0.12, 0.02)
2-level (Detect/ND)	**Difference in least square means (95% CI) [c]**			
Chlorpyrifos (Detect vs ND)	-0.01 (-0.09, 0.07)	0.02 (-0.06, 0.10)	0.01 (-0.06, 0.07)	0.02 (-0.04, 0.08)
Phorate (Detect vs ND)	-0.04 (-0.14, 0.06)	0.02 (-0.08, 0.12)	-0.03 (-0.11, 0.06)	0.04 (-0.03, 0.11)
	Iron deficient			
	9 months			
OP insecticide	**Wave V (n=30)**	**CCT (n=29)**	**IPI III-V (n=29)**	**IPI-I-III (n=29)**
Continuous	**β (95% CI) for OP [b]**			
# OP Detects	-0.00 (-0.06, 0.06)	-0.03 (-0.09, 0.04)	-0.01 (-0.05, 0.04)	-0.02 (-0.06, 0.02)
Log-Naled	0.00 (-0.10, 0.11)	0.01 (-0.10, 0.11)	0.01 (-0.07, 0.08)	0.00 (-0.06, 0.07)
3-level (High/Med./ND)	**Difference in least square means (95% CI) [c]**			
Methamidophos (High vs ND)	0.03 (-0.21, 0.27)	0.06 (-0.19, 0.32)	-0.01 (-0.19, 0.16)	0.09 (-0.07, 0.24)
Methamidophos (Med. vs ND)	-0.02 (-0.25, 0.27)	0.03 (-0.21, 0.26)	0.02 (-0.14, 0.18)	0.02 (-0.13, 0.16)
Trichlorfon (High vs ND)	0.07 (-0.16, 0.31)	-0.04 (-0.29, 0.22)	0.03 (-0.14, 0.20)	-0.06 (-0.21, 0.08)
Trichlorfon (Med. vs ND)	-0.04 (-0.29, 0.21)	-0.05 (-0.32, 0.22)	-0.02 (-0.20, 0.17)	-0.01 (-0.17, 0.15)
2-level (Detect/ND)	**Difference in least square means (95% CI) [c]**			
Chlorpyrifos (Detect vs ND)	-0.05 (-0.24, 0.13)	-0.14 (-0.33, 0.06)	-0.05 (-0.18, 0.08)	-0.08 (-0.20, 0.04)
Phorate (Detect vs ND)	-0.08 (-0.35, 0.20)	-0.18 (-0.47, 0.12)	-0.07 (-0.26, 0.12)	-0.11 (-0.29, 0.07)

[a] Models adjusted for sex and age at testing
[b] Estimated change in ABR latency per 1 unit increase in OP
[c] Difference in mean ABR latency
High/Medium/ND cut-offs (ng/mL): methamidophos >18.2/1.5-18.2/ND; trichlorfon >1.7/ 0.4-1.7/ND; chlorpyrifos ≥0.04/ND; phorate ≥1.8/ND
Abbreviations: ABR, auditory brainstem response; CCT, central conduction time; IPI, inter-peak interval

Table IV.A3, continued

	Iron sufficient			
	18months			
OP insecticide	**Wave V (n=101)**	**CCT (n=78)**	**IPI III-V (n=78)**	**IPI-I-III (n=78)**
Continuous	**β (95% CI) for OP [b]**			
# OP Detects	0.00 (-0.02, 0.03)	0.00 (-0.02, 0.03)	0.00 (-0.02, 0.02)	0.00 (-0.02, 0.02)
Log-Naled	-0.00 (-0.04, 0.04)	-0.01 (-0.05, 0.03)	-0.01 (-0.04, 0.03)	0.00 (-0.03, 0.03)
3-level (High/Med./ND)	**Difference in least square means (95% CI) [c]**			
Methamidophos (High vs ND)	0.02 (-0.08, 0.12)	0.04 (-0.06, 0.14)	0.04 (-0.04, 0.12)	0.00 (-0.07, 0.08)
Methamidophos (Med. vs ND)	-0.04 (-0.14, 0.06)	-0.02 (-0.12, 0.09)	-0.01 (-0.10, 0.08)	0.00 (-0.08, 0.08)
Trichlorfon (High vs ND)	-0.00 (-0.10, 0.09)	0.03 (-0.07, 0.13)	0.01 (-0.08, 0.09)	0.02 (-0.06, 0.09)
Trichlorfon (Med. vs ND)	0.04 (-0.06, 0.14)	0.01 (-0.09, 0.11)	0.03 (-0.05, 0.12)	-0.03 (-0.10, 0.05)
2-level (Detect/ND)	**Difference in least square means (95% CI) [c]**			
Chlorpyrifos (Detect vs ND)	0.01 (-0.08, 0.10)	-0.01 (-0.10, 0.07)	0.02 (-0.06, 0.09)	-0.03 (-0.09, 0.04)
Phorate (Detect vs ND)	0.04 (-0.06, 0.14)	0.02 (-0.09, 0.12)	0.02 (-0.06, 0.11)	-0.01 (-0.09, 0.07)
	Iron deficient			
	18 months			
OP insecticide	**Wave V (n=16)**	**CCT (n=16)**	**IPI III-V (n=16)**	**IPI-I-III (n=16)**
Continuous	**β (95% CI) for OP [b]**			
# OP Detects	-0.01 (-0.07, 0.05)	-0.02 (-0.09, 0.06)	-0.01 (-0.06, 0.04)	-0.00 (-0.05, 0.04)
Log-Naled	0.02 (-0.08, 0.12)	0.05 (-0.07, 0.17)	0.04 (-0.04, 0.13)	0.02 (-0.06, 0.10)
3-level (High/Med./ND)	**Difference in least square means (95% CI) [c]**			
Methamidophos (High vs ND)	0.11 (-0.14, 0.36)	0.17 (-0.11, 0.45)	0.07 (-0.13, 0.28)	0.11 (-0.07, 0.29)
Methamidophos (Med. vs ND)	0.05 (-0.18, 0.28)	0.12 (-0.14, 0.37)	0.09 (-0.10, 0.28)	0.04 (-0.13, 0.20)
Trichlorfon (High vs ND)	-0.00 (-0.25, 0.25)	-0.03 (-0.32, 0.26)	0.00 (-0.21, 0.21)	-0.04 (-0.22, 0.14)
Trichlorfon (Med. vs ND)	-0.07 (-0.31, 0.17)	-0.06 (-0.35, 0.22)	0.02 (-0.18, 0.22)	-0.09 (-0.26, 0.08)
2-level (Detect/ND)	**Difference in least square means (95% CI) [c]**			
Chlorpyrifos (Detect vs ND)	-0.07 (-0.26, 0.12)	-0.17 (-0.39, 0.05)	-0.13 † (-0.29, 0.02)	-0.03 (-0.17, 0.11)
Phorate (Detect vs ND)	-0.10 (-0.38, 0.18)	-0.17 (-0.48, 0.14)	-0.08 (-0.30, 0.13)	-0.07 (-0.27, 0.12)

[a] Models adjusted for sex and age at testing
[b] Estimated change in ABR latency per 1 unit increase in OP
[c] Difference in mean ABR latency
High/Medium/ND cut-offs (ng/mL): methamidophos >18.2/1.5-18.2/ND; trichlorfon >1.7/ 0.4-1.7/ND; chlorpyrifos ≥0.04/ND; phorate ≥1.8/ND
Abbreviations: ABR, auditory brainstem response; CCT, central conduction time; IPI, inter-peak interval
†p<0.10

Table IV.A4. Adjusted [a] longitudinal change/difference in ABR latencies by OP exposure

	ABR outcome					
	6 weeks		9 months		18 months	
OP insecticide	Wave V (n=181)	CCT (n=180)	Wave V (n=165)	CCT (n=147)	Wave V (n=126)	CCT (n=94)
Continuous	**β (95% CI) for OP [b]**					
# OP Detects	0.01 (-0.01, 0.03)	0.01 (-0.01, 0.03)	-0.02 (-0.04, 0.01)	-0.01 (-0.03, 0.01)	-0.00 (-0.03, 0.02)	-0.00 (-0.03, 0.02)
Log-Naled	0.00 (-0.0.3, 0.04)	0.01 (-0.02, 0.05)	-0.00 (-0.04, 0.03)	0.01 (-0.02, 0.05)	-0.00 (-0.04, 0.04)	-0.00 (-0.04, 0.04)
3-level (High/Med./ND)	**Difference in least square means (95% CI) [c]**					
Methamidophos (High vs ND)	0.05 (-0.03, 0.14)	0.07 † (-0.01, 0.15)	0.00 (-0.08, 0.08)	0.06 (-0.03, 0.14)	0.02 (-0.07, 0.11)	0.05 (-0.04, 0.14)
Methamidophos (Med. vs ND)	-0.03 (-0.11, 0.06)	-0.01 (-0.09, 0.07)	-0.02 (-0.10, 0.06)	-0.03 (-0.11, 0.06)	-0.03 (-0.12, 0.06)	0.01 (-0.09, 0.10)
Trichlorfon (High vs ND)	0.04 (-0.04, 0.13)	0.02 (-0.06, 0.10)	-0.02 (0.11, 0.06)	-0.06 (-0.10, 0.07)	-0.02 (-0.11, 0.07)	0.01 (-0.08, 0.11)
Trichlorfon (Med. vs ND)	-0.00 (-0.09, 0.08)	-0.00 (-0.08, 0.08)	-0.04 (-0.13, 0.05)	-0.04 (-0.13, 0.04)	0.01 (-0.08, 0.11)	-0.01 (-0.11, 0.08)
2-level (Detect/ND)	**Difference in least square means (95% CI) [c]**					
Chlorpyrifos (Detect vs ND)	-0.02 (-0.09, 0.05)	-0.01 (-0.08, 0.06)	-0.02 (-0.10, 0.05)	-0.01 (-0.08, 0.07)	0.02 (-0.07, 0.11)	0.05 (-0.04, 0.14)
Phorate (Detect vs ND)	0.03 (-0.06, 0.11)	0.03 (-0.05, 0.12)	-0.06 (-0.15, 0.04)	-0.02 (-0.11, 0.08)	-0.03 (-0.12, 0.06)	0.01 (-0.09, 0.10)

[a] Models adjusted for sex, age at testing, cord ferritin, and head circumference
[b] Estimated change in ABR latency per 1 unit increase in OP
[c] Difference in mean ABR latency
High/Medium/ND cut-offs (ng/mL): methamidophos >18.2/1.5-18.2/ND; trichlorfon >1.7/ 0.4-1.7/ND; chlorpyrifos ≥0.04/ND; phorate ≥1.8/ND
Abbreviations: ABR, auditory brainstem response; CCT, central conduction time
†p<0.10, *p<0.05

Table IV.A4, continued

	ABR outcome					
	6 weeks		9 months		18 months	
OP insecticide	IPI III-V (n=181)	IPI-I-III (n=180)	IPI III-V (n=147)	IPI-I-III (n=148)	IPI III-V (n=94)	IPI-I-III (n=94)
Continuous	**β (95% CI) for OP [b]**					
# OP Detects	0.00 (-0.01, 0.02)	0.01 (-0.01, 0.02)	-0.01 (-0.03, 0.01)	0.00 (-0.01, 0.02)	-0.00 (-0.02, 0.02)	0.00 (-0.02, 0.02)
Log-Naled	0.01 (-0.02, 0.03)	0.01 (-0.02, 0.03)	0.00 (-0.02, 0.03)	0.01 (-0.01, 0.04)	-0.00 (-0.03, 0.03)	0.00 (-0.03, 0.03)
3-level (High/Med./ND)	**Difference in least square means (95% CI) [c]**					
Methamidophos (High vs ND)	0.02 (-0.04, 0.08)	0.05 (-0.01, 0.10)	0.00 (-0.06, 0.07)	0.05 † (-0.00, 0.11)	0.04 (-0.04, 0.11)	0.02 (-0.05, 0.08)
Methamidophos (Med. vs ND)	-0.02 (-0.08, 0.04)	0.00 (-0.05, 0.06)	-0.01 (-0.08, 0.05)	-0.01 (-0.07,0.05)	0.01 (-0.07, 0.09)	0.00 (-0.07, 0.07)
Trichlorfon (High vs ND)	0.05 (-0.01, 0.11)	-0.03 (-0.09, 0.03)	0.00 (-0.06, 0.07)	-0.01 (-0.07, 0.05)	-0.00 (-0.08, 0.07)	0.01 (-0.06, 0.08)
Trichlorfon (Med. vs ND)	0.05 (-0.02, 0.11)	-0.05 † (-0.10, 0.01)	0.00 (-0.07, 0.07)	-0.04 (-0.10, 0.02)	0.02 (-0.05, 0.10)	-0.04 (-0.10, 0.03)
2-level (Detect/ND)	**Difference in least square means (95% CI) [c]**					
Chlorpyrifos (Detect vs ND)	0.00 (-0.05, 0.05)	-0.01 (-0.06, 0.04)	-0.01 (-0.06, 0.05)	0.00 (0.05, 0.05)	-0.01 (-0.07, 0.06)	-0.03 (-0.09, 0.03)
Phorate (Detect vs ND)	0.04 (-0.03, 0.10)	-0.00 (-0.06, 0.06)	-0.04 (-0.11, 0.04)	0.02 (-0.05, 0.09)	-0.00 (-0.08, 0.08)	-0.01 (-0.08, 0.06)

[a] Models adjusted for sex, age at testing, cord ferritin, and head circumference
[b] Estimated change in ABR latency per 1 unit increase in OP
[c] Difference in mean ABR latency
High/Medium/ND cut-offs (ng/mL): methamidophos >18.2/1.5-18.2/ND; trichlorfon >1.7/ 0.4-1.7/ND; chlorpyrifos ≥0.04/ND; phorate ≥1.8/ND
Abbreviations: ABR, auditory brainstem response; IPI, inter-peak interval
†p<0.10, *p<0.05

CHAPTER V

DISCUSSION

Summary of research findings

This dissertation work provides important information about the levels of prenatal exposure of Chinese infants to many classes of pesticides. It also gives valuable insight into the effects of prenatal OP insecticide exposure on infant neurodevelopment, specifically the motor and sensory tracts of the brain.

In the first aim we characterized the prenatal exposure of Chinese newborns to 96 pesticides of all classes in cord blood and identified predictors of that exposure. We found evidence of prenatal exposure to 75 pesticides; neonates were exposed to detectable levels of 15 pesticides, on average. OP and PYR levels were several-fold higher than those reported in cord blood in the U.S. Season of birth was the strongest and most consistent predictor of cord pesticides. The total number of pesticides detected, the total insecticides, and the total OPs, PYRs, and fungicides detected were all higher in the cord blood of infants born in the summer months of June to September, compared to those born between October and December. When pesticides were analyzed on an individual basis we also found that pesticides varied significantly by season/month. It is likely that the increased pesticide levels in cord blood in the

summer correspond with maternal consumption of fresh food grown during farming season in Zhejiang (June to September) (M. Zhang, et al., 2013).

In the second aim we investigated the effects of prenatal OP exposure on infant motor function using the PDMS-2 and INFANIB. Here we found that prenatal naled and chlorpyrifos exposure was associated with decreased motor function in 9-month-old infants. For naled, we observed negative effects on fine motor outcomes only, while chlorpyrifos was associated with deficits in both gross and fine motor function. Additionally, odds of abnormal gross motor quotient (GMQ) at 9 months was nearly three times higher in infants prenatally exposed to chlorpyrifos, compared to those who were not exposed. No statistically significant associations were observed between prenatal OP exposure and infant INFANIB scores at either time point. Trends revealed that infants with more OP exposure had lower INFANIB scores and higher odds of an abnormal score at 6 weeks of age, but results were not statistically significant. Interestingly, sex-specific analyses revealed that girls seemed to be more sensitive to the effects of prenatal OP exposure on motor outcomes than boys.

In the third aim we examined the effects of prenatal OP exposure on infant visual and auditory function, using grating VA and ABR, respectively. We found that infants prenatally exposed to chlorpyrifos had lower grating VA scores at 9 months of age, compared to unexposed infants. Differences by sex were not evident early in the study period, however by 18 months of age, exposed girls seemed to have consistently lower VA scores, while exposed boys actually had significantly higher VA. We did not observe any statistically significant

associations between prenatal OP exposure and infant ABR latencies, though trends revealed that infants exposed to methamidophos had consistently longer ABR latencies, indicating slower auditory signal transmission. In a supplementary analysis, we additionally found reduced 9-month head circumferences in infants exposed to chlorpyrifos and phorate prenatally.

In this dissertation work, we present associations between prenatal exposure to certain OPs and deficits in global motor function, visual acuity, and head circumference. Chlorpyrifos was associated with statistically significant deficits in both global motor function and visual acuity, as well as reduced head circumference, in 9-month-old infants. Naled was significantly associated with deficits in 9-month fine motor function. Methamidophos was consistently associated with deficits in 9- month global motor and slower auditory signal transmission across the three time points, yet results never reached statistical significance. Phorate was not associated with any of the neurodevelopmental outcomes examined here, but was significantly associated with reduced head circumference at 9 months of age. Of these commonly used OPs, only chlorpyrifos had been studied for neurodevelopmental effects in humans prior to this study.

Interestingly, many of our results seem to be sexually dimorphic. Sex-specific differences in early-life motor function and visual acuity are common. Female infants tend to have better fine motor functioning, while males excel at gross motor tasks (Piek, Gasson, Barrett, & Case, 2002). Sex-specific effects of OPs on motor outcomes have been reported in rodent (ACGIH, 2013; Dam,

Seidler, & Slotkin, 2000) and human studies (Y. Zhang, et al., 2014), yet no clear patterns emerge. VA maturation is also thought to be accelerated in girls, compared to boys, during the first six months of life (Makrides, Neumann, & Gibson, 2001), though sex-specific effects of OPs on visual related outcomes have not previously been examined.

Synthesis of proposed mechanisms

Low doses of OPs have been reported to disrupt a host of neuronal processes such as DNA synthesis, neuronal replication and differentiation, axon formation, synaptogenesis, apoptosis, neural circuit formation and signaling, and to enhance reactive oxygen species formation (Garcia, Seidler, & Slotkin, 2005; Slotkin, 2004). In chapters three and four, we explored a variety of possible mechanisms for how prenatal OP exposure may affect either motor or visual outcomes in infancy. Synthesis of those mechanisms revealed that several may be relevant across both motor and visual outcomes; those findings are synthesized here.

One of the ways chlorpyrifos has been shown to induce neurotoxicity is via the disruption of the development and function of 5HT receptor circuits in the brain (Slotkin & Seidler, 2007b). Dysfunction of the 5HT signaling systems has been associated with a variety of behavioral abnormalities in rats (Slotkin & Seidler, 2007b). These 5HT serotonergic pathways play an important role in the maturation of spinal locomotor networks (De Felice, Scattoni, Ricceri, &

Calamandrei, 2015) and in the plasticity and long-term potentiation of the visual cortex synaptic plasticity in infancy (Edagawa, Saito, & Abe, 2001). Therefore, it is possible that disruption of the timing of 5HT circuit development, as result of prenatal OP exposure, could potentially have negative consequences on early-life motor and visual functions.

Laboratory studies also reveal that OPs can elicit neurotoxicity by disrupting glial cell development and function in the brain (Garcia, Seidler, Crumpton, & Slotkin, 2001; Garcia, Seidler, Qiao, & Slotkin, 2002; Garcia, Seidler, & Slotkin, 2003; Zurich, Honegger, Schilter, Costa, & Monnet-Tschudi, 2004). Glial cells (astrocytes, oligodendrocytes, microglia) provide important support functions for neurons and alterations in their development can affect synaptic plasticity, architectural modeling, and myelination processes (Garcia, et al., 2005). Rodent studies have shown that early life exposure to chlorpyrifos and diazinon, during the onset of myelination, elicits deficits in expression of genes involved in oligodendrocyte function and myelination processes (Garcia, et al., 2001; Garcia, et al., 2002; Garcia, et al., 2003; Slotkin & Seidler, 2007a). Gains in motor function and mobility during infancy correspond to increases in corticospinal tract myelination (Carlson, 2014; Dąmbska & Wisniewski, 1999). Similarly, improvements in grating VA also directly correlate with increasing myelination and maturation of the visual pathway (Tau & Peterson, 2010). Both of these tracts begin myelinating late in pregnancy (Carlson, 2014; Dąmbska & Wisniewski, 1999; Tau & Peterson, 2010). Therefore, prenatal OP exposure that occurs during the onset of corticospinal or visual tract myelination may also have

the potential to disrupt motor- and visual-related outcomes.

Neurotoxicological effects at the cellular level are difficult to determine in infants and children; however, recent brain imaging studies provide some clues about long-term morphologic changes that may be associated with prenatal OP exposure. A recent magnetic resonance imaging (MRI) study revealed that prenatal chlorpyrifos exposure was associated with enlargements of white matter in the superior temporal (MST), posterior middle temporal (MT), and inferior postcentral gyri, and the frontal gyrus, gyrus rectus, cuneus, and precuneus in the right hemisphere of 5-11-year-old children (Rauh, et al., 2012). The authors speculated that the increased white matter was likely representative of glial scarring, a common response to cellular injury, and an effect which has been observed in rodents following early-life chlorpyrifos exposure (Roy, Sharma, Seidler, & Slotkin, 2005). As previously mentioned, OPs have been found to disrupt glial cell formation and function in the developing brain (Garcia, et al., 2005). It may, therefore, be plausible that this early-life glial cell dysfunction could eventually manifest in childhood as scarring and white matter enlargement.

Several of the brain areas that are reportedly affected by prenatal chlorpryifos exposure (Rauh, et al., 2012) may also be associated with motor and visual function. The precuneus has cortical connections with the premotor area and supplementary motor areas of the frontal cortex (Cavanna & Trimble, 2006). An MRI study revealed that grey matter volume in the right precuneus of 12-month-olds is directly correlated with 18-month fine motor function (Sanz-Cortes, et al., 2010). Interestingly, the precuneus has also been implicated in ASD and

ADHD. Children with ASD and ADHD have significantly higher levels of brain activation in the precuneus, compared to controls (Christakou, et al., 2013).

The cuneus is important in modulating signaling between the primary visual cortex and the extrastriate visual cortices (Vanni, Tanskanen, Seppa, Uutela, & Hari, 2001), and the MST and MT are on the dorsal stream of the extrastriate visual cortex (Blumberg & Kreiman, 2010). MT and MST are involved in processing visuospatial information, including the detection of motion, position, and depth perception (Born & Bradley, 2005; Maunsell, 1995). Lesions in the MT have been associated with visual deficits in monkeys (Born & Bradley, 2005).

It is unclear whether the precuneus directly affects infant motor function, or whether the cuneus, MST, or MT might directly influence grating acuity in infancy. However, given that prenatal chlorpyrifos has been associated with increased white matter or glial scarring in the precuneus, cuneus, MST and MT in children, it is possible that this may be one mechanism by which OPs could possibly affect motor or visual function.

Impact and innovation

This is the largest and most comprehensive exposure assessment of prenatal pesticide exposure anywhere in the world to date. We measured prenatal exposure to 96 pesticides of all classes in the cord blood of a potentially highly exposed population of infants. Measuring pesticide parent compounds

directly is preferable to other, more commonly used, exposure assessment methods because it provides direct evidence of fetal exposure (Barr, et al., 1999; Munoz-Quezada, et al., 2013), may more accurately reflect the available dose (Needham, Ashley, & Patterson, 1995), and informs regulatory agencies' risk assessments.

This work also presents the first analyses of the health effects of naled, methamidophos, trichlorfon, and phorate in non-occupationally exposed human populations. Naled is used in public health vector-control campaigns for controlling diseases that are spread by adult mosquitoes, and has recently been employed in Florida to combat Zika virus (U.S. EPA, 2016; EXTOXNET, 1993a; Frieden, Schuchat, & Petersen, 2016). Methamidophos, trichlorfon, and phorate are applied widely to control insects and rootworms in field and root crops, livestock, and pine forests, and parasites in fish and domestic animals (EXTOXNET, 1993b, 1993c, 1995). Therefore, this work provides some vital initial insights about the neurodevelopmental effects of prenatal exposure to these commonly used, yet grossly understudied, OPs.

The motor assessment utilized here presents a significant advantage over the assessments used in previous studies. The PDMS-2 is sensitive to changes in both gross and fine motor function, and is considered a gold standard for predicting motor development (Liao, et al., 2012). This test provides a more comprehensive view of overall motor function in infancy than the motor portions of the Bayley (Engel, et al., 2011; Eskenazi, et al., 2010; Eskenazi, et al., 2007; Rauh, et al., 2006) or Brazleton (Engel, et al., 2007; Young, et al., 2005) tests

that have been employed previously. The secondary motor test, the INFANIB, further provides an additional unique lens through which to examine motor, and more specifically neuromotor, development.

Pesticides have largely not been studied for their visual and auditory effects in infants and children. The current work represents the largest study of prenatal pesticide exposure and VA and ABR, to date. There are only two former studies and both were limited by small sample sizes and imprecise exposure estimates (Handal, Harlow, Breilh, & Lozoff, 2008; Sturza, et al., 2016). The grating VA and ABR tests provide a non-invasive way of measuring the maturation of the visual and auditory pathways throughout infancy. Additionally, these tests were assessed at three time points over the course of infancy. This longitudinal study design provides a more comprehensive view of sensory development than the two previous studies.

Recommendations for future research

This data-rich cohort offers many opportunities for further study. An obvious next step should be to examine the remaining pesticides for associations with infant neurodevelopment in this cohort. There is little to no information regarding the health effects of exposure to the majority of the pesticides we measured in the general population, and even less is known in regards to developmental neurotoxicity (Grandjean & Landrigan, 2014). Second, while this dissertation was an important step in assessing the associations of individual

pesticides with the neurodevelopmental outcomes of interest, it will be important for future work to address the health effects of exposure to pesticide mixtures. Environmental pollutants are regulated as single chemicals, so it is imperative to study them as such, like we did here, yet is also important to study them as humans are exposed in the environment, as mixtures. In this cohort, infants were exposed to 15 pesticides on average, not one pesticide at a time. Therefore, the next step of this research should be to employ modeling techniques that allow for the consideration of exposure to mixtures of pesticides. Similarly, future work in this cohort should also involve further exploration of pesticide-iron deficiency interactions.

There are a number of factors that should be considered in the design of future, related studies. New studies should strive to include multiple time points for the exposure assessment. The biggest weakness of the current study was that exposure was only measured at one time point. Multiple time points are needed to get an accurate assessment of exposure to the non-persistent pesticides on the market today (Eskenazi, et al., 2007; Grandjean & Landrigan, 2014). Ideally, future studies should include maternal pesticide levels during pregnancy and infant/child levels at multiple time points over the study period. Longer follow-up times are also warranted to determine if small, subclinical effects observed during infancy are later manifested as neurological disorders in childhood. This is important because brain functions develop sequentially, the full effects of early neurotoxic damage from pesticides might not become apparent until later in childhood (Grandjean & Landrigan, 2014)

Since the current study was not originally designed to examine environmental exposures, it is missing some secondary information that would be helpful to understanding exposure and vulnerability in this population. Collection of additional lifestyle data could potentially enrich future studies. For example, information on residential pesticide use, maternal diet during pregnancy, proximity to agricultural areas, and additional detail about parental occupation would improve insights into pesticide exposures in this population. Similarly, genetic polymorphisms are believed to play an important role in the metabolism of pesticides in humans, thereby influencing individual susceptibility (Engel, et al., 2011). Incorporating genotyping analyses into future studies could provide additional important insights. Additional considerations for susceptible infants should include those who are born pre-term or low birth weight (LBW). LBW or premature infants are more likely to experience developmental delays (Chan, Johnson, Leaf, & Vollmer, 2016; Spittle & Treyvaud, 2016) and it is important to consider the effects of prenatal pesticide exposure on neurodevelopmental outcomes in these vulnerable groups.

Overall conclusions

This dissertation provides important information about the prenatal exposure of Chinese infants to pesticides, as well as providing critical insights about the effects of prenatal OP exposure on infant neurodevelopment, specifically the motor and sensory pathways of the brain. This study provides the

first information regarding the developmental neurotoxicity of four OPs previously unstudied in humans. We report deficits in fine and gross motor function, visual acuity, and head circumference in OP-exposed infants at 9 months. Early motor skill acquisition in infancy provides the basis for cognitive and socio-emotional development in childhood (Clearfield, 2004, 2011), as well as providing the foundation for non-verbal communication (Bhat, Galloway, & Landa, 2012). Similarly, visual and auditory system development in infancy is crucial for the development of language and other forms of communication, as well as reading skills in childhood (Algarin, Peirano, Garrido, Pizarro, & Lozoff, 2003; Chonchaiya, et al., 2013). Therefore, delays or altered timing of motor or sensory systems maturation, possibly as a result of prenatal OP exposure, could potentially have detrimental long-term effects on learning or other cognitive functions in childhood. Even small, subclinical changes, that may seem negligible on an individual level, could have potentially detrimental effects at the population level. Given the nearly ubiquitous exposure among the general population, the dearth of information regarding the developmental neurotoxicity of so many of the pesticides on the market today, the unique vulnerability of fetal and infant brains, and the potential for long-term effects on cognition and behavior, this work is both relevant and necessary.

References

ACGIH. (2013). *Documentation of the TLVs and BEIs with other world wide occupational exposure values*. Cincinnati, Ohio: American Conference of Governmental Industrial Hygienists.

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*(2), 217-223.

Barr, D. B., Barr, J. R., Driskell, W. J., Hill, R. H., Jr., Ashley, D. L., Needham, L. L., et al. (1999). Strategies for biological monitoring of exposure for contemporary-use pesticides. *Toxicol Ind Health, 15*(1-2), 168-179.

Bhat, A. N., Galloway, J. C., & Landa, R. J. (2012). Relation between early motor delay and later communication delay in infants at risk for autism. *Infant Behav Dev, 35*(4), 838-846.

Blumberg, J., & Kreiman, G. (2010). How cortical neurons help us see: visual recognition in the human brain. *J Clin Invest, 120*(9), 3054-3063.

Born, R. T., & Bradley, D. C. (2005). Structure and function of visual area MT. *Annu Rev Neurosci, 28*, 157-189.

Carlson, B. M. (2014). *Human Embryology and Developmental Biology* (5th ed.): Elsevier.

Cavanna, A. E., & Trimble, M. R. (2006). The precuneus: a review of its functional anatomy and behavioural correlates. *Brain, 129*(Pt 3), 564-583.

Chan, S. H., Johnson, M. J., Leaf, A. A., & Vollmer, B. (2016). Nutrition and neurodevelopmental outcomes in preterm infants: a systematic review. *Acta Paediatr, 105*(6), 587-599.

Chonchaiya, W., Tardif, T., Mai, X., Xu, L., Li, M., Kaciroti, N., et al. (2013). Developmental trends in auditory processing can provide early predictions of language acquisition in young infants. *Dev Sci, 16*(2), 159-172.

Christakou, A., Murphy, C. M., Chantiluke, K., Cubillo, A. I., Smith, A. B., Giampietro, V., et al. (2013). Disorder-specific functional abnormalities during sustained attention in youth with Attention Deficit Hyperactivity Disorder (ADHD) and with autism. *Mol Psychiatry, 18*(2), 236-244.

Clearfield, M. W. (2004). The role of crawling and walking experience in infant spatial memory. *J Exp Child Psychol, 89*(3), 214-241.

Clearfield, M. W. (2011). Learning to walk changes infants' social interactions. *Infant Behav Dev, 34*(1), 15-25.

Dam, K., Seidler, F. J., & Slotkin, T. A. (2000). Chlorpyrifos exposure during a critical neonatal period elicits gender-selective deficits in the development of coordination skills and locomotor activity. *Brain Res Dev Brain Res, 121*(2), 179-187.

Dąmbska, M., & Wisniewski, K. E. (1999). *Normal and Pathologic Development of the Human Brain and Spinal Cord*. London, England: John LIbby & Co. Ltd.

De Felice, A., Scattoni, M. L., Ricceri, L., & Calamandrei, G. (2015). Prenatal exposure to a common organophosphate insecticide delays motor

development in a mouse model of idiopathic autism. *PLoS One, 10*(3), e0121663.
Edagawa, Y., Saito, H., & Abe, K. (2001). Endogenous serotonin contributes to a developmental decrease in long-term potentiation in the rat visual cortex. *J Neurosci, 21*(5), 1532-1537.
Engel, S. M., Berkowitz, G. S., Barr, D. B., Teitelbaum, S. L., Siskind, J., Meisel, S. J., et al. (2007). Prenatal organophosphate metabolite and organochlorine levels and performance on the Brazelton Neonatal Behavioral Assessment Scale in a multiethnic pregnancy cohort. *Am J Epidemiol, 165*(12), 1397-1404.
Engel, S. M., Wetmur, J., Chen, J., Zhu, C., Barr, D. B., Canfield, R. L., et al. (2011). Prenatal exposure to organophosphates, paraoxonase 1, and cognitive development in childhood. *Environ Health Perspect, 119*(8), 1182-1188.
U.S. EPA. (2016). Naled for Mosquito Control. Retrieved October 10, 2016, from https://www.epa.gov/mosquitocontrol/naled-mosquito-control
Eskenazi, B., Huen, K., Marks, A., Harley, K. G., Bradman, A., Barr, D. B., et al. (2010). PON1 and neurodevelopment in children from the CHAMACOS study exposed to organophosphate pesticides in utero. *Environ Health Perspect, 118*(12), 1775-1781.
Eskenazi, B., Marks, A. R., Bradman, A., Harley, K., Barr, D. B., Johnson, C., et al. (2007). Organophosphate pesticide exposure and neurodevelopment in young Mexican-American children. *Environ Health Perspect, 115*(5), 792-798.
EXTOXNET. (1993a). Naled pesticide information profile. Retrieved October 31, 2016, from www.pmep.cce.cornell.edu/profiles/extoxnet/metiram-propoxur/naled-ext.html
EXTOXNET. (1993b). Phorate pesticide information profile. *Extension Toxicology Network* Retrieved March 1, 2017, from www.pmep.cce.cornell.edu/profiles/extoxnet/metiram-propoxur/phorate-ext.html
EXTOXNET. (1993c). Trichlorfon pesticide information profile. Retrieved March 1, 2017, from www.pmep.cce.cornell.edu/profiles/extoxnet/pyrethrins-ziram/trichlorfon-ext.html
EXTOXNET. (1995). Methamidophos pesticide information profile. Retrieved March 1, 2016, from www.pmep.cce.cornell.edu/profiles/extoxnet/haloxyfop-methylparathion/methamidophos-ext.html
Frieden, T. R., Schuchat, A., & Petersen, L. R. (2016). Zika Virus 6 Months Later. *JAMA, 316*(14), 1443-1444.
Garcia, S. J., Seidler, F. J., Crumpton, T. L., & Slotkin, T. A. (2001). Does the developmental neurotoxicity of chlorpyrifos involve glial targets? Macromolecule synthesis, adenylyl cyclase signaling, nuclear transcription factors, and formation of reactive oxygen in C6 glioma cells. *Brain Res, 891*(1-2), 54-68.

Garcia, S. J., Seidler, F. J., Qiao, D., & Slotkin, T. A. (2002). Chlorpyrifos targets developing glia: effects on glial fibrillary acidic protein. *Brain Res Dev Brain Res, 133*(2), 151-161.

Garcia, S. J., Seidler, F. J., & Slotkin, T. A. (2003). Developmental neurotoxicity elicited by prenatal or postnatal chlorpyrifos exposure: effects on neurospecific proteins indicate changing vulnerabilities. *Environ Health Perspect, 111*(3), 297-303.

Garcia, S. J., Seidler, F. J., & Slotkin, T. A. (2005). Developmental neurotoxicity of chlorpyrifos: targeting glial cells. *Environ Toxicol Pharmacol, 19*(3), 455-461.

Grandjean, P., & Landrigan, P. J. (2014). Neurobehavioural effects of developmental toxicity. *Lancet Neurol, 13*(3), 330-338.

Handal, A. J., Harlow, S. D., Breilh, J., & Lozoff, B. (2008). Occupational exposure to pesticides during pregnancy and neurobehavioral development of infants and toddlers. *Epidemiology, 19*(6), 851-859.

Kim, M., Kang, S. K., Yee, B., Shim, S. Y., & Chung, M. (2016). Paternal involvement and early infant neurodevelopment: the mediation role of maternal parenting stress. *BMC Pediatr, 16*(1), 212.

Liao, W., Wen, E. Y., Li, C., Chang, Q., Lv, K. L., Yang, W., et al. (2012). Predicting neurodevelopmental outcomes for at-risk infants: reliability and predictive validity using a Chinese version of the INFANIB at 3, 7 and 10 months. *BMC Pediatr, 12*, 72.

Makrides, M., Neumann, M. A., & Gibson, R. A. (2001). Perinatal characteristics may influence the outcome of visual acuity. *Lipids, 36*(9), 897-900.

Maunsell, J. H. (1995). The brain's visual world: representation of visual targets in cerebral cortex. *Science, 270*(5237), 764-769.

Moller, E. L., Nikolic, M., Majdandzic, M., & Bogels, S. M. (2016). Associations between maternal and paternal parenting behaviors, anxiety and its precursors in early childhood: A meta-analysis. *Clin Psychol Rev, 45*, 17-33.

Munoz-Quezada, M. T., Lucero, B. A., Barr, D. B., Steenland, K., Levy, K., Ryan, P. B., et al. (2013). Neurodevelopmental effects in children associated with exposure to organophosphate pesticides: a systematic review. *Neurotoxicology, 39*, 158-168.

Needham, L. L., Ashley, D. L., & Patterson, D. G., Jr. (1995). Case studies of the use of biomarkers to assess exposures. *Toxicol Lett, 82-83*, 373-378.

Piek, J. P., Gasson, N., Barrett, N., & Case, I. (2002). Limb and gender differences in the development of coordination in early infancy. *Hum Mov Sci, 21*(5-6), 621-639.

Rauh, V. A., Garfinkel, R., Perera, F. P., Andrews, H. F., Hoepner, L., Barr, D. B., et al. (2006). Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. *Pediatrics, 118*(6), e1845-1859.

Rauh, V. A., Perera, F. P., Horton, M. K., Whyatt, R. M., Bansal, R., Hao, X., et al. (2012). Brain anomalies in children exposed prenatally to a common

organophosphate pesticide. *Proc Natl Acad Sci U S A, 109*(20), 7871-7876.
Roy, T. S., Sharma, V., Seidler, F. J., & Slotkin, T. A. (2005). Quantitative morphological assessment reveals neuronal and glial deficits in hippocampus after a brief subtoxic exposure to chlorpyrifos in neonatal rats. *Brain Res Dev Brain Res, 155*(1), 71-80.
Sanz-Cortes, M., Padilla, N., Falcon, C., Bargallo, N., Figueras, F., Botet, F., et al. (2010). *Analysis of brain structure by MRI voxel based morphometry (VBM) and neurodevelopment in preterm born infants with and without IUGR*. Paper presented at the 20th World Congress on Ultrasound in Obstetrics and Gynecology.
Sethna, V., Pote, I., Wang, S., Gudbrandsen, M., Blasi, A., McCusker, C., et al. (2016). Mother-infant interactions and regional brain volumes in infancy: an MRI study. *Brain Struct Funct*.
Slotkin, T. A. (2004). Guidelines for developmental neurotoxicity and their impact on organophosphate pesticides: a personal view from an academic perspective. *Neurotoxicology, 25*(4), 631-640.
Slotkin, T. A., & Seidler, F. J. (2007a). Comparative developmental neurotoxicity of organophosphates in vivo: transcriptional responses of pathways for brain cell development, cell signaling, cytotoxicity and neurotransmitter systems. *Brain Res Bull, 72*(4-6), 232-274.
Slotkin, T. A., & Seidler, F. J. (2007b). Prenatal chlorpyrifos exposure elicits presynaptic serotonergic and dopaminergic hyperactivity at adolescence: critical periods for regional and sex-selective effects. *Reprod Toxicol, 23*(3), 421-427.
Spittle, A., & Treyvaud, K. (2016). The role of early developmental intervention to influence neurobehavioral outcomes of children born preterm. *Semin Perinatol, 40*(8), 542-548.
Sturza, J., Silver, M. K., Xu, L., Li, M., Mai, X., Xia, Y., et al. (2016). Prenatal exposure to multiple pesticides is associated with auditory brainstem response at 9months in a cohort study of Chinese infants. *Environ Int, 92-93*, 478-485.
Tau, G. Z., & Peterson, B. S. (2010). Normal development of brain circuits. *Neuropsychopharmacology, 35*(1), 147-168.
Vanni, S., Tanskanen, T., Seppa, M., Uutela, K., & Hari, R. (2001). Coinciding early activation of the human primary visual cortex and anteromedial cuneus. *Proc Natl Acad Sci U S A, 98*(5), 2776-2780.
Young, J. G., Eskenazi, B., Gladstone, E. A., Bradman, A., Pedersen, L., Johnson, C., et al. (2005). Association between in utero organophosphate pesticide exposure and abnormal reflexes in neonates. *Neurotoxicology, 26*(2), 199-209.
Zhang, M., Fang, X., Zhou, L., Su, L., Zheng, J., Jin, M., et al. (2013). Pesticide poisoning in Zhejiang, China: a retrospective analysis of adult cases registration by occupational disease surveillance and reporting systems from 2006 to 2010. *BMJ Open, 3*(11), e003510.

Zhang, Y., Han, S., Liang, D., Shi, X., Wang, F., Liu, W., et al. (2014). Prenatal exposure to organophosphate pesticides and neurobehavioral development of neonates: a birth cohort study in shenyang, china. *PLoS One, 9*(2), e88491.

Zurich, M. G., Honegger, P., Schilter, B., Costa, L. G., & Monnet-Tschudi, F. (2004). Involvement of glial cells in the neurotoxicity of parathion and chlorpyrifos. *Toxicol Appl Pharmacol, 201*(2), 97-104.