HHS Public Access

Author manuscript

Environ Int. Author manuscript; available in PMC 2018 September 01.

Published in final edited form as:

Environ Int. 2017 September ; 106: 248–256. doi:10.1016/j.envint.2017.05.015.

Prenatal naled and chlorpyrifos exposure is associated with deficits in infant motor function in a cohort of Chinese infants

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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 here 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).

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

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CONFLICT OF INTEREST

All authors declare they have no conflicts of interest

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. 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.

Keywords

Organophosphate; Pesticide; Peabody; PDMS-2; Motor development; Neurodevelopment

1. 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 and Bao 2013; U.S.EPA 2011; Zhang et al. 2011), at up to 5 times the global average (Huang et al. 2001; Zhang et al. 2014). Organophosphate insecticides (OPs) account for more than a third of all insecticide use in China (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 (Huang et al. 2001; NPIC 2010; U.S.CDC 2016). Additionally, warming temperatures have seen a surge in the transmission of mosquito-borne infectious diseases (Bai et al. 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 et al. 2005). Fetal susceptibility is further increased by the fact that OPs can cross the placenta (Bradman et al. 2003; Koutroulakis et al. 2014; Tzatzarakis et al. 2009). Associations have been reported between prenatal exposures to OPs and deficits in IQ (Bouchard et al. 2011; Engel et al. 2011; Rauh et al. 2011), and increases in autism spectrum (Shelton et al. 2014), attention deficit-hyperactivity (Marks et al. 2010; Rauh et al. 2006), and pervasive developmental disorder (Eskenazi et al. 2007; 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 and Murphy 2013). Delays in meeting early motor milestones may be indicative of a developmental disorder (De Felice et al. 2015; Noritz and 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; 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 (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 et al. 2008) and reduced motor speed and coordination in 6- to 8-year-olds (Harari et al. 2010). With the exception of the Rauh, et al., 2006 study, which only looked at chlorpyrifos, the existing body of work is largely limited by the use of imprecise exposure assessments, such as maternal non-specific urinary metabolites or self-reported occupational exposure during pregnancy. These limited exposure assessments make it difficult to accurately define exposure and attribute observed effects to specific OPs, thus restricting the utility of the findings for regulatory considerations. Additionally, with the exception of a few well-studied OPs, such as chlorpyrifos, many of the OPs in use today have not been studied for neurodevelopmental effects in non-occupationally exposed populations. Therefore, the current study sought to investigate associations between prenatal exposure to multiple OP insecticides, measured directly in umbilical cord blood, and many of which have been understudied in humans, and gross and fine motor function in infancy.

2. METHODS

2.1 Population

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.

2.2 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).

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

The Peabody Developmental Motor Scales (PDMS-2) (Folio and Fewell 1983, 2000) 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 and 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 and 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 and Fewell 1983). PDMS-2 data was available for 199 infants.

2.4 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 PDMS-2 testing, cord ferritin, gestational age, birth weight, maternal education and occupation, family income, and season of PDMS-2 testing.

Linear mixed models (LMM) were used to evaluate associations between cord OP exposures and PDMS raw scores (subtest [reflexes, stationary, locomotion, grasping, V-M] and composite [GM, FM, TM]), as well as motor quotients (GMQ, FMQ, TMQ), at 6 weeks and 9 months. 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 PDMS-2 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. 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. We also completed a sensitivity analysis to determine if 9-month iron status was significantly affecting 9-month motor outcomes by including it in the model. Finally, to investigate differences in the effect of prenatal OP exposure on infant motor function by sex, we carried out additional LMM modeling stratified by infant sex.

3. RESULTS

All infants were born to term (≥37 weeks) and of healthy birth weight (≥2.5 kg). Pertinent sample characteristics are presented in Table 1. Additional household and parental characteristics of the study sample have been previously published (Silver et al. 2016). Infants with missing exposure or outcome data did not significantly differ from those with complete data (Table A.1). Levels of OP exposure for those with and without PDMS data are compared in Table A.2. 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 3.0 (1.6) and ranged from 1 to 8. Distributions of detectable OPs and their LODs are shown in Table 2.

There were no significant associations between any of the OPs measured and PDMS outcomes at 6 weeks (Table A.3). Adjusted LMM results for PDMS outcomes at 9 months are shown in Table 3. Log-naled was associated with deficits in fine motor function (Table 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 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.

The addition of 9-month iron status to the models did not substantially impact the results. Associations between naled and V-M, FM, and FMQ were slightly attenuated; β (p-value): −0.50 (p=0.05), −0.76 (p=0.05), and −0.87 (p=0.09), respectively. Associations between chlorpyrifos and PDMS-2 outcomes were somewhat strengthened, with the exception of V-M which was slightly attenuated; β (p-value): −0.48 (p=0.03), −0.73 (p=0.08), −2.23 (p=0.009), −0.98 (p=0.01), −1.87 (p=0.009), −3.77 (p=0.004), −2.84 (p<0.001), −6.71 (p<0.001), −3.05 (p=0.002), −2.47 (p=0.02), −3.12 (p=0.001), for reflexes, stationary, locomotion, grasping, V-M, GM, FM, TM, GMQ, FMQ, and TMQ, respectively.

Sex-stratified LMM results are shown in Table A.4 (6 weeks) and Figure 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 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.

4. 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. 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 and Fewell 2000; Snider et al. 2009).

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; Yan et al. 2009; 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 (U.S.EPA 2016).

It has been employed for routine mosquito control in the U.S. and following hurricanes and floods (U.S.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 et al. 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 (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; Rauh et al. 2006; 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; 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 (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 (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 and Karami-Mohajeri 2012; Eddleston et al. 2008; Kamanyire and 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 and 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 et al. 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 et al. 2004; Torres-Altoro et al. 2011). 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 and Seidler 2007a). Chlorpyrifos has also been found to induce aberrant striatal dopamine neurotransmission, possibly via the dysregulation of cyclin-dependent kinase 5 (Cdk5) activity (Torres-Altoro et al. 2011). Monoaminergic pathways play an important role in the maturation of spinal locomotor networks (De Felice et al. 2015) and in the mediation of motor control (Philibin et al. 2011). 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 et al. 2001; Garcia et al. 2002; Garcia et al. 2003; Zurich et al. 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 and Seidler 2007b). Gains in motor function and mobility during infancy correspond to increases in corticospinal tract myelination, a process that begins in late pregnancy (Carlson 2014; Dambska and 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 et al. 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 (Rauh et al. 2006); other studies used non-specific urinary metabolites during pregnancy (Engel et al. 2007; Engel et al. 2011; Eskenazi et al. 2007; Eskenazi et al. 2010; Young et al. 2005; 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. For example, 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), using the PDMS-2, which is sensitive to changes in both gross and fine motor function. The PDMS-2, which has been called a "gold standard" for predicting motor development (Liao et al. 2012), offers an advantage over the motor assessments used in previous studies, which have largely used the motor portions of larger tests of overall development such as the Bayley (Engel et al. 2011; Eskenazi et al. 2007; Eskenazi et al. 2010; Rauh et al. 2006) or Brazleton (Engel et al. 2007; Young et al. 2005). Therefore, the longitudinal design, as well as the use of the PDMS-2, gives a more comprehensive view of overall motor function in infancy than previous studies.

5. 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

The authors are grateful to the families who participated in the study and the doctors and nurses at Zhejiang University Children's Hospital for their dedication and assistance with the project.

FUNDING

This study was supported 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).

ABBREVIATIONS

OP	organophosphate
PDMS-2	Peabody developmental motor scales- 2nd edition
V-M	visual-motor integration subtest
GM	gross motor score
FM	fine motor score
TM	total motor score
GMQ	gross motor quotient
FMQ	fine motor quotient
TMQ	total motor quotient

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HIGHLIGHTS

- Chinese infants prenatally exposed to organophosphate insecticides were tested for motor function at 6-weeks and 9-months
- Prenatal naled and chlorpyrifos exposure was associated with statistically significant deficits in 9-month motor outcomes.
- Prenatal organophosphate exposure may affect infant motor skill development.

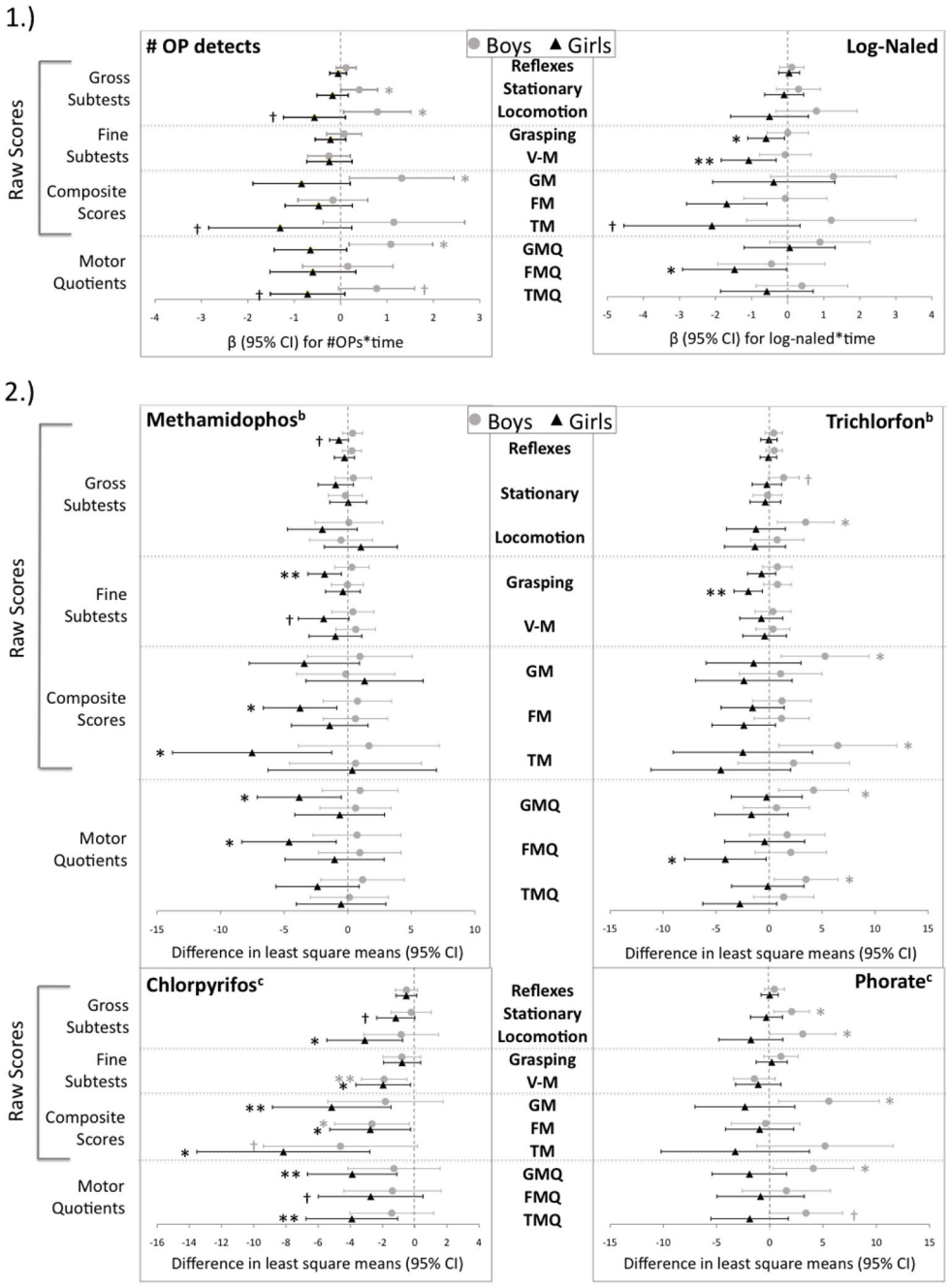

Figure 1.

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

1.) Estimated change in 9-month motor score per 1 unit increase in OP exposure

2.) Difference in mean 9-month 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

$^{\dagger}p<0.10$, $^{*}p<0.05$, $^{**}p<0.01$

Table 1

Study sample characteristics

Variable	N	Mean (SD)
Age (days) at 6 week testing	231	43.0 (5.1)
Age (days) at 9 month testing	218	283.3 (10.6)
Gestational age (weeks)	233	39.6 (1.0)
Birth weight (kg)	233	3.4 (0.4)
		N (%)
Sex (male)	233	121 (51.9)
Low cord ferritin (≤75 μg/L)	218	42 (19.3)

Table 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

Abbreviations: ND, non-detect; DEDTP, diethyldithiophosphate; DMTP, dimethylthiophosphate, TCPY, 3,5,6-trichloro-2-pyridinol; DMDTP, dimethyldithiophosphate

Table 3

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

	Raw Subtest Scores					Composite Raw Scores			Motor Quotients		
OP Insecticide	**Reflexes (n=182)**	**Stationary (n=188)**	**Locomotion (n=187)**	**Grasping (n=191)**	**V-M (n=192)**	**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	0.03 (−0.10, 0.17)	0.08 (−0.18, 0.33)	0.04 (−0.44–0.53)	−0.08 (−0.32, 0.17)	−0.21 (−0.54, 0.13)	0.12 (−0.64, 0.88)	−0.29 (−0.80, 0.23)	−0.14 (−1.21, 0.93)	0.11 (−0.48, 0.69)	−0.23 (−0.89, 0.43)	−0.03 (−0.60, 0.53)
Log-Naled	0.08 (−0.14, 0.30)	0.07 (−0.33, 0.47)	0.06 (−0.71, 0.83)	−0.30 (−0.68, 0.08)	−0.55 *(−1.07, −0.30)	0.36 (−0.84, 1.55)	−0.85 *(−1.65, −0.06)	−0.53 (−2.20, 1.15)	0.44 (−0.48, 1.36)	−0.90 †(−1.92, 0.12)	−0.10 (−0.99, 0.79)
3-level (High/Med./ND)	*Difference in least square means (95% CI)[c]*										
Methamidophos (High vs ND)	−0.12 (−0.66, 0.41)	−0.31 (−1.29, 0.67)	−1.03 (−2.91, 0.85)	−0.70 (−1.63, 0.23)	−0.55 (−1.83, 0.73)	−1.30 (−4.24, 1.65)	−1.25 (−3.21, 0.71)	−2.77 (−6.89, 1.36)	−1.32 (−3.51, 0.87)	−1.67 (−4.18, 0.85)	−0.62 (−2.90, 1.67)
Methamidophos (Med. vs ND)	0.06 (−0.48, 0.59)	−0.13 (−1.10, 0.84)	0.13 (−1.73, 1.99)	−0.13 (−1.06, 0.79)	−0.01 (−1.28, 1.26)	0.41 (−2.53, 3.35)	−0.18 (−2.11, 1.77)	0.46 (−3.64, 4.57)	0.07 (−2.12, 2.25)	0.25 (−2.25, 2.75)	−0.23 (−2.52, 2.06)
Trichlorfon (High vs ND)	0.28 (−0.27, 0.83)	0.59 (−0.40, 1.58)	1.19 (−0.71, 3.09)	0.08 (−0.87, 1.03)	−0.10 (−1.40, 1.21)	2.12 (−0.88, 5.12)	−0.05 (−2.06, 1.96)	2.39 (−1.84, 6.61)	2.07 (−0.24, 4.38)	0.70 (−1.87, 3.26)	1.77 (−0.46, 4.00)
Trichlorfon (Med. vs ND)	0.30 (−0.23, 0.84)	−0.17 (−1.14, 0.80)	−0.06 (−1.92, 1.81)	−0.54 (−1.46, 0.39)	0.07 (−1.34, 1.21)	−0.28 (3.20, 2.64)	−0.60 (−2.55, 1.35)	−0.75 (−4.84, 3.33)	−0.26 (−2.53, 2.01)	−0.97 (−3.47, 1.54)	−0.50 (−2.67–1.67)
2-level (Detect/ND)	*Difference in least square means (95% CI)[c]*										
Chlorpyrifos (Detect vs ND)	−0.50 *(−0.96, 0.04)	−0.67 (−1.52, 0.18)	−1.98 *(−3.62, −0.35)	−0.80 †(−1.61, 0.01)	−1.91 ***(−3.01, −0.81)	−3.49 **(−6.03, −0.95)	−2.71 **(−4.40, −1.02)	−6.29 ***(−9.83, −2.75)	−2.56 *(−4.53, −0.59)	−2.04 †(−4.23, 0.15)	−2.59 **(−4.49, −0.70)
Phorate (Detect vs ND)	0.22 (−0.39, 0.82)	0.70 (−0.40, 1.80)	0.49 (−1.64, 2.61)	0.66 (−0.40, 1.73)	−1.01 (−2.45, 0.44)	1.28 (−2.00, 4.57)	−0.38 (−2.62, 1.86)	1.07 (−3.59, 5.73)	0.79 (−1.74, 3.33)	0.51 (−2.35, 3.37)	0.69 (−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: 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

† p<0.10,

* p<0.05,

** p<0.01,

*** p<0.001