



## Full length article

# Organophosphorus pesticide exposure and neurobehavioral performance in Latino children living in an orchard community



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## ABSTRACT

Children living in agricultural communities have a greater risk from pesticides due to para-occupational pathways. The goal of this study was to assess the impact of exposure to organophosphorus pesticides on the neurobehavioral performance of school-aged Latino children over time. Two exposure measures were used to estimate children's pesticide exposure: parent's occupation (agricultural or non-agricultural) and organophosphate residues in home carpet dust samples. During 2008–2011, 206 school-aged children completed a battery of neurobehavioral tests two times, approximately one year apart. The associations between both exposure measures and neurobehavioral performance were examined. Pesticide residues were detected in dust samples from both agricultural and non-agricultural homes, however, pesticides were detected more frequently and in higher concentrations in agricultural homes compared to non-agricultural homes. Although few differences were found between agricultural and non-agricultural children at both visits, deficits in learning from the first visit to the second visit, or less improvement, was found in agricultural children relative to non-agricultural children. These differences were significant for the Divided Attention and Purdue Pegboard tests. These findings are consistent with previous research showing deficits in motor function. A summary measure of organophosphate residues was not associated with neurobehavioral performance. Results from this study indicate that children in agricultural communities are at increased risk from pesticides as a result of a parent working in agricultural. Our findings suggest that organophosphate exposure may be associated with deficits in learning on neurobehavioral performance, particularly in tests of with motor function. In spite of regulatory phasing out of organophosphates in the U.S., we still see elevated levels and higher detection rates of several organophosphates in agricultural households than non-agricultural households, albeit lower levels than prior studies.

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## 1. Introduction

Organophosphorus insecticides (OPs) are commonly used to control pests in agricultural settings, both in the United States and globally. OPs impact humans by interfering with the transmission of nerve impulses by blocking the normal breakdown of the neurotransmitter, acetylcholine, through cholinesterase inhibition. Through this mechanism of action, these insecticides have known neurotoxic properties, particularly in children [1,2]. Children are considered to be more vulnerable than adults to the toxic effects of

OPs because of physiological differences such as immature metabolism and elimination systems [3].

Although use of these pesticides has been reduced and restricted in the United States, they are still applied to some agricultural crops [4]. Recent evidence suggests that low-level exposure to OPs during childhood and adolescence may have adverse consequences on neurologic development [5–9]. Also recent longitudinal birth cohort studies assessing prenatal exposures have shown deficits in cognition [10–12]. However, there are still questions about neurologic development deficits related to chronic exposures over time and the timing of exposure during critical windows of development.

Research has indicated that families living in agricultural communities have a greater risk from OPs due to chronic exposures than the general population [13–16], additionally children have greater exposure due to their behaviors such as crawling on the

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floor and more frequent hand-to-mouth activity [17]. Home carpet dust samples are commonly used to assess OP levels in the home. Prior studies have shown that pesticide levels in home dust are positively associated with the proximity of homes to pesticide-treated fields and with para-occupational pathways, agricultural workers inadvertently bringing pesticide residues into the home on their clothes, boots, skin and hair [13–15,18–20].

OPs have the potential to adversely affect the health and neurodevelopment of children living in agricultural communities where they are applied in the orchards and fields. Thus, the purpose of this study is to investigate associations between OP exposure and neurobehavioral performance in school-aged Latino children living in an orchard community. Furthermore, the possibility of potential learning deficits in children due to the impact of pesticide exposure was investigated. In the study, we compared two neurobehavioral evaluations performed approximately 12 months apart.

## 2. Methods

### 2.1. Study participants and design

This longitudinal study was conducted in an orchard community in the Pacific Northwest where OPs are commonly applied. Many families in this community work in the orchards or fruit packing houses. Children between the ages of 5 and 12 were recruited during a three-year period between 2008 and 2010. Recruitment occurred through word-of-mouth, at school, and at community events where a booth was set up with information explaining the study. Only one child per household was eligible to participate in the study. For each child, data were collected at two

time points approximately one year apart. At both time points, children completed a neurobehavioral test battery, parents completed a series of questionnaires, and dust samples were collected from the homes. All test materials were administered to children and parents in their preferred language, either Spanish or English. The study was approved by the Institutional Review Board at Oregon Health and Science University.

### 2.2. OP exposures

Parent's occupation and OP residues in carpet dust samples were used to characterize exposure to pesticides in children. Children that had at least one parent currently working in agriculture were classified as agricultural and children that had neither parent working in agriculture during the previous five years were classified as non-agricultural.

#### 2.2.1. Questionnaires

Parents completed a series of questionnaires to collect demographic information, occupational history, pesticide use at work and at home [14,21]. In addition, the Home Observation for Measurement of the Environment (HOME) survey [22] was administered through interviews with the mother in the home, established HOME scores were calculated and higher HOME scores indicate a more enriched home environment; the School-age Child Behavior Checklist (CBCL) [23] was also completed, three established scores were generated from the CBCL (total behavior problems, internalizing problems and externalizing problems), higher scores indicate more behavioral and emotional problems. Children completed the Short Acculturation Scale for Hispanic Youth (SASH-Y) survey [24], established acculturation scores were

**Table 1**  
Neurobehavioral test, function measured, and outcome examined.

Neurobehavioral tests and descriptions	Function	Outcome (measured unit)
1. Digit span Recall of number sequences	Memory & attention	Forward score (maximum digits) Reverse score (maximum digits)
2. Finger tapping Tapping with preferred and non-preferred hand for 20 s	Response speed & coordination	Preferred and non-preferred hand (number of taps)
3. Match-to-sample 15 stimuli are shown for 3 s (10 × 10 matrix of blocks) Identify correct stimuli from 3 choices	Visual Memory	Average latency for correct choice (ms) Number correct
4. Symbol-digit Digits are paired with symbols in matrix Match numbers to the symbols from the key	Processing Speed	Average latency of response for correct match (ms)
5. Continuous performance Different shapes and targets shown rapidly for 4 min, subjects are instructed to press a key when a target is presented	Attention	Percent correct hits (%) D-prime, measures how well the participant discriminates non-targets from target
6. Divided attention Tapping with preferred and non-preferred hand (control) Tapping while reciting the birthday song with preferred and non-preferred hand (distraction)	Divided attention	Control, tapping with no song Preferred and non-preferred hand (number of taps) Distraction, reciting birthday song
7. Object Memory <sup>a</sup> Show 16 objects and asked to recall name	Recall & recognition memory	Preferred and non-preferred hand (number of taps) Preferred and non-preferred hand (number of times sang song) <sup>†</sup> Utilization Immediate recall of objects
8. Purdue Pegboard <sup>b</sup> Place small pegs in holes during two 30 s trials with each hand and both hands	Dexterity	Recognition of target and non-target items Preferred hand (average number of pegs) Non-preferred hand (average number of pegs)
9. Visual motor integration <sup>b</sup> Total score for correct line segments	Hand-eye coordination	Both hands (average number of pegs) Figure copying score
10. Name writing <sup>c</sup> Time it takes to write name	Visuomotor & fine-motor agility	Preferred hand, latency (s) Non-preferred hand, latency (s)

Abbreviations: ms, milliseconds; s, seconds.

<sup>a</sup> [8,32].

<sup>b</sup> Pediatric environmental neurobehavioral test battery [33].

<sup>c</sup> [34,35].

<sup>†</sup> Individually administered tests were selected from previous studies.

generated. Higher scores indicate higher acculturation to the U.S. society.

2.2.2. Dust collection

Dust samples were collected at both time points from homes with carpets and analyzed for four OPs: azinphos-methyl, phosmet, malathion, and chlorpyrifos. These pesticides were selected because they were commonly applied to orchard crops at the time of data collection [25]. Standard protocols were used to collect and analyze the samples [13]. Briefly, samples were collected from carpet in the main entrance or living area of the home using a high-volume small surface sampler (HVS4, CS3, Inc.). An area of 122 cm by 122 cm was divided longitudinally into three strips with masking tape for sampling. The HVS4 was placed at the first strip and pushed from the beginning to the end of the strip. Each strip was sampled back and forth four times [13]. Samples were analyzed by gas chromatography analysis with a mass selective detector in selected ion monitoring mode for the four targeted OPs [13,18]. Pesticide residues below the lower limits of detection (LOD) were assigned a value one-half the appropriate LOD [26]. To assess the health effects of all four OP exposures cumulatively, the raw OP levels were converted to molar equivalents. They were then multiplied by the specific relative potency factors, and summed to create OP molar totals for Time 1 and Time 2. Relative potency factors for the oral exposure routes of OPs were used. Azinphos-methyl was set at 1.0 and the other three OP potency factors were relatively adjusted (phosmet, 0.2; malathion, 0.003; and chlorpyrifos, 0.6) in order to be able to sum the OPs together [27]. This approach was selected because the OPs act through a common mode of toxic action and can be predicted by an additive toxicity approach that can be estimated from the sum of the individual toxic potencies of each individual compound [28]. OP concentration results were reported as both nanograms of pesticide per gram dust (ng/g) for each specific pesticide and

nanomoles of pesticide per gram dust (nmol/g) for the OP molar total.

2.3. Neurobehavioral tests

To assess the long-term health effects of OP exposure on these children, neurobehavioral data were collected using a battery of six computer-based tests from the Behavioral Assessment and Research System (BARS) and four individually administered tests (Table 1). The BARS has been used successfully in farmworker populations [29,30] and with Hispanic children [31]. Neurobehavioral testing was administered individually to each child in a private room at a community center or library.

2.4. Statistical analysis

We first summarized demographic characteristics between the non-agricultural and agricultural groups using chi-square and the Wilcoxon–Mann–Whitney tests to examine differences between the groups. Descriptive statistics for each type of OP by group were calculated using the Wilcoxon–Mann–Whitney test.

Potential confounders were selected for these analyses based on results from the literature [36–38] and are listed in Table 2. Separate multivariate linear regression models were built using the two exposure measures (parent’s occupation and OP residues in carpet dust samples) and identified confounders in a predictive model for each neurobehavioral outcome. These models were run both for cross-sectional analyses of neurobehavioral outcomes at the first visit, the second visit, and the difference in outcome between the second visit and first visit to examine the change in test performance over time. Covariates were retained in the final models if the level of significance for the association with the neurobehavioral outcomes was <0.10 in the bivariate analyses and if the beta coefficient for the independent exposure measures was

**Table 2**  
Demographic characteristics of Latino non-agricultural and agricultural children, N=215.

Variables	Non-agricultural (n = 60)		Agricultural (n = 155)		p-Value <sup>a</sup>
	Mean (SD)	Range	Mean (SD)	Range	
Age, years	8.3 (2.1)	5–12	8.5 (2.2)	5–13	0.60
Education, years	3.0 (2.1)	0–7	2.8 (2.2)	0–7	0.54
Mother’s education, years	8.9 (4.3)	0–14	8.1 (3.3)	0–15	0.07
Father’s education, years	7.6 (4.5)	0–14	6.8 (3.1)	0–17	0.21
Acculturation score <sup>a</sup>	33.5 (8.0)	15–55	28.0 (6.2)	11–38	<0.001
HOME score <sup>b</sup>	49.8 (4.3)	41–57	46.5 (6.5)	22–58	<0.01
CBCL score-total <sup>c</sup>	18.7 (12.8)	0–47	20.6 (14.0)	0–59	0.45
Internalizing <sup>c</sup>	5.2 (4.4)	0–19	5.8 (4.8)	0–19	0.52
Externalizing <sup>c</sup>	4.8 (4.3)	0–20	5.5 (5.1)	0–22	0.41
	N (%)		N (%)		p-Value <sup>**</sup>
Male gender	31 (52%)		87 (56%)		0.73
Home close to field <sup>d</sup>	16 (26)		83 (54)		<0.001
Computer use at home	48 (80)		88 (57)		<0.01
Housing type					
Apartment/duplex	53 (88)		101 (65)		<0.01
Cabin/trailer	7 (12)		54 (35)		
Home ownership					
Owned	32 (53)		48 (31)		<0.001
Rented	27 (45)		52 (34)		
Employer provided	1 (2)		53 (35)		

<sup>a</sup> Acculturation scores—short acculturation scale for Hispanic youth, measures level of acculturation of Hispanic children, scores range from 12 to 60. Higher scores indicating higher acculturation to U.S. society.

<sup>b</sup> HOME scores—home observation for measurement of the environment, measures stimulation and support of a child’s home environment, scores range from 0 to 59. Higher total scores indicate a more enriched home environment.

<sup>c</sup> CBCL score—child behavior checklist, parents rate their child’s behavior on multiple syndrome scales, two broad scales (internalizing and externalizing) and a total behavior score. Higher scores indicate more deviant behavior.

<sup>d</sup> Home close to field—question asked: do you live next to or within an agricultural field or orchard?

<sup>a</sup> Wilcoxon–Mann–Whitney test.

<sup>\*\*</sup> Chi-square test.

changed by more than 10% with the addition of the covariate in the model. A priori, we decided to retain child's age and gender in the cross-sectional models. Child's age, gender and CBCL external score were included in all the cross-sectional models for parent's occupation. Child's age, gender and acculturation score were included in all the cross-sectional models for OP molar total. HOME scores were included in the longitudinal models. Other confounders were included in the models depending on the specific test, which included acculturation score, mother's education, and child's home computer use. Confounders were checked for collinearity in the adjusted regression models using variance inflation factors.

The results of the longitudinal associations are presented as the adjusted change in neurobehavioral performance ( $\beta$ ) associated with increased exposure: (1) agricultural children compared to non-agricultural children and (2) increase in change in OP molar total from Time 1 to Time 2. To standardize the direction of the multivariate associations (so that higher values always indicated better performance), values for Match-to-Sample (latency), Symbol Digit (latency), Object Memory (utilization) and Name Writing (latency) tests were inverted. All beta coefficients of  $\beta < 0$  indicate a negative association. In other words, increased exposure is associated with poorer neurobehavioral performance. For longitudinal associations,  $\beta < 0$  indicates a negative association between increased exposure and less improvement in performance from Time 1 to Time 2. *P*-values  $< 0.05$  were considered statistically significant and all statistical analyses were performed using SAS 9.3 (SAS Institute, Cary NC).

### 3. Results

#### 3.1. Demographic results

A total of 328 children participated in the study. Due to the low number of non-Latino agricultural families participating (7%), and large differences in demographics between Latino and non-Latino children, (e.g., parent's education levels), only data from children of Latino families were included in this analysis ( $N = 215$ ). There was

no difference between non-agricultural and agricultural children in age, gender, years of education of child, mother, and father, and CBCL scores (Table 2). A greater percentage of agricultural children lived next to or within an agricultural field or orchard and in small homes or cabins provided by the grower/owner operator than non-agricultural children. Non-agricultural children had significantly higher acculturation and HOME scores and more non-agricultural children reported using computers at their home. There were no differences between the groups on language spoken at home, number of moves the family had made in the past 12 months, and number of pesticides used in the home, with a mean of one product used for both groups (not shown).

#### 3.2. OP dust measures

A total of 311 dust samples from 183 homes were collected at Time 1 and Time 2 between 2008 and 2011 and analyzed for the four OPs. The LOD and detection frequencies are provided in Table 3. Dust samples were only collected from homes with carpeting (84%). Detectable levels of OP residues were found in 100% of all homes from either Time 1 or Time 2 samples (96% and 99%, respectively). However, a greater number of OP residues were detected in agricultural homes compared to non-agricultural homes (Table 3). Malathion was most frequently detected and azinphos-methyl was detected the least frequently. Higher median levels of OP residues were found in agricultural homes at both time points with the exception of phosmet at Time 2 (Table 3). When assessing within-household residue levels between Time 1 and Time 2, malathion levels increased significantly and phosmet levels significantly decreased for agricultural households from Time 1 to Time 2. Chlorpyrifos levels increased significantly for non-agricultural households from Time 1 to Time 2.

#### 3.3. Neurobehavioral performance among non-agricultural and agricultural children

The proportion of children completing all of the neurobehavioral tests was greater for non-agricultural children

**Table 3**  
Summary statistics for organophosphate residues in carpet dust<sup>#</sup> by collection time between non-agricultural and agricultural households.

OPs	Non-agricultural households					Agricultural Households					Median OP residue level comparisons	
	Time 1 (n=47)		Time 2 (n=35)		Time 1 vs. Time 2 p-Value*	Time 1 (n=116)		Time 2 (n=111)		Time 1 vs. Time 2 p-Value*	Time 1 p-Value**	Time 2 p-Value**
	Detected <sup>a</sup> (%)	Median, ng/g (IQR)	Detected <sup>a</sup> (%)	Median, ng/g (IQR)		Detected <sup>a</sup> (%)	Median, ng/g (IQR)	Detected <sup>a</sup> (%)	Median, ng/g (IQR)			
Azinphos-methyl	44%	2.5 (2.0–8.0)	31%	2.5 (2.0–8.0)	0.92	67%	8.5 (2.5–30.0)	59%	9.0 (2.5–30.0)	0.33	<0.01	<0.01
Phosmet	65%	4.0 (2.0–10.0)	56%	3.0 (1.0–7.0)	0.38	80%	9.5 (2.5–26.0)	66%	5.0 (1.0–16.0)	<0.01	0.01	0.12
Malathion	83%	9.0 (5.0–14.0)	89%	12.0 (6.0–27.0)	0.07	90%	15.0 (7.0–59.0)	92%	32.0 (9.0–120.0)	<0.01	<0.01	0.01
Chlorpyrifos	75%	7.0 (2.0–12.0)	83%	14.0 (5.0–22.0)	<0.01	88%	18.0 (10.0–38.0)	92%	19.0 (9.0–44.0)	0.21	<0.01	0.04
OP molar total <sup>b</sup>	–	0.04 (0.02–0.05)	–	0.05 (0.02–0.08)	0.31	–	0.09 (0.04–0.20)	–	0.10 (0.04–0.18)	0.90	<0.01	0.01

Abbreviation: OPs, organophosphorus insecticides; IQR, interquartile range. –, not defined for molar total.

<sup>a</sup> Percentage of frequency of detection (%) for each organophosphate measured above the LOD. LOD levels: 2008 LOD: 4.0 ng/g dust for all organophosphates; 2009 LOD: 2.0 ng/g dust for all organophosphates; 2010 LODs: 2.0 ng/g dust for phosmet, malathion, and chlorpyrifos; 5.0 ng/g dust for azinphos-methyl; 2011 LODs: 4.0 ng/g dust for azinphos-methyl and chlorpyrifos, 2.0 ng/g dust for Phosmet and malathion.

<sup>b</sup> Sum of four organophosphorus insecticides molar concentrations multiplied by relative potency factors for oral exposure route, reported as nanomoles per gram dust (nmol/g).

\* Wilcoxon signed rank test. Only those households with samples for both visits were included in analyses (non-agricultural:  $N = 31$  and agricultural:  $N = 95$ ).

\*\* Wilcoxon–Mann–Whitney test.

<sup>#</sup> Values below the limit of detection (LOD) were imputed with half the LOD before computing summary statistics.

compared to agricultural children at both Time 1 (67% vs. 56%,  $\chi^2=1.8$ ,  $p=0.18$ ) and Time 2 (71% vs. 57%,  $\chi^2=2.3$ ,  $p=0.13$ ), although these differences were not significant. The Digit Span test (reverse) was the test the children had the most difficulty with at both time points, Table 4. Both groups showed improvement in performance on all outcome measures at Time 2 compared to Time 1, except for the latency scores on the Match-to-Sample test for the non-agricultural group (Table 4).

3.4. Associations between exposure measures and neurobehavioral performance

In the cross-sectional analysis of neurobehavioral test outcomes at Time 1, agricultural children had poorer performance on two outcome measures of attention, Digit Span (forward), and Divided Attention (times sang song while tapping with preferred hand) while controlling for confounders [ $\beta=-0.34$  and  $\beta=-0.24$ , respectively] and performed better on Object Memory (utilization),  $\beta=0.65$ , Table 5. At Time 2, agricultural children had poorer performance on the Divided Attention test (times sang song while tapping with preferred and non-preferred hands) [ $\beta=-0.37$  and  $\beta=-0.48$ , respectively]. In the longitudinal analysis, agricultural children had less improvement from Time 1 to Time 2 on 14 out of the 24 outcome measures, however only four outcome measures were significant, Divided Attention test (taps, preferred hand; taps with song, preferred hand; and times sang song while tapping with

non-preferred hand), and Purdue Pegboard test (non-preferred hand), Table 5.

OP molar total was only significantly associated with one outcome measure at Time 2, Continuous Performance (correct hits) (data not shown).

4. Discussion

The relationship between neurobehavioral performance and measures of potential exposure to OPs among school-aged Latino children in an orchard community in the Pacific Northwest was examined. Two measures were used to assess potential exposures to OPs among children: parent’s occupation and OP residues in carpet dust samples.

4.1. Comparing OP measures in dust samples

Children whose parent(s) worked in agriculture had significantly more detectable OPs in the home and higher OP residues than children whose parent(s) did not work in agriculture indicating greater opportunities for exposure to pesticides. These results support the findings from prior studies which indicate that agricultural workers bring pesticide residues home from their workplace, increasing the risk from pesticides to children in that household. Although measures were higher in agricultural homes, prior data collected in this community found OP residues in home

Table 4 Mean scores and standard deviations for neurobehavioral outcomes between non-agricultural and agricultural children.

Neurobehavioral test (possible score range)	Non-agricultural children				Agricultural children			
	N=55		N=41		N=151		N=129	
	N	Time 1 mean (SD)	N	Time 2 mean (SD)	N	Time 1 mean (SD)	N	Time 2 mean (SD)
Completed tests (1–10) <sup>a</sup>	37	9.11 (1.8)	29	9.32 (1.5)	85	8.83 (2.0)	74	8.75 (2.0)
Digit span test								
Forward (3–9)	52	4.9 (1.2)	40	5.1 (1.2)	133	4.6 (1)	115	4.8 (1.1)
Reverse (3–9)	42	3.5 (0.8)	36	3.8 (1.0)	99	3.6 (0.9)	95	3.9 (.9)
Finger tapping test								
Taps, P	54	64.8 (12.8)	40	72.2 (11.7)	136	67.1 (12.8)	115	72.0 (12.9)
Taps, NP	54	54.9 (12.0)	40	61.9 (13.2)	136	57.9 (11.8)	115	63.0 (11.9)
Match-to-sample test								
Correct (0–15)	51	12.4 (2.0)	40	13.2 (1.6)	128	12.8 (2.0)	115	12.8 (2.1)
Latency, ms <sup>b</sup>	51	4100 (900)	40	4260 (1520)	128	4150 (990)	115	4000 (900)
Symbol-digit test								
Latency, ms <sup>b</sup>	52	4150 (1560)	39	3710 (2270)	131	4020 (1730)	111	3360 (1350)
Continuous performance test								
Correct hits (%)	52	89% (14)	40	90% (10)	133	91% (13)	110	92% (12)
d prime	52	3.0 (1.0)	40	3.1 (1.0)	133	3.1 (0.96)	110	3.3 (1.0)
Divided attention test								
Taps, P	51	54.2 (10.8)	40	71.9 (11.9)	129	55.8 (10.5)	110	59.3 [11]
Taps, NP	51	47.3 (10.0)	40	62.2 (13.2)	129	48.9 (10.0)	109	53.0 (9.6)
Taps with song, P	51	45.5 (12.7)	40	60.8 (10.5)	129	47.2 (12.0)	110	53.0 (11.1)
Taps with song, NP	51	42.7 (9.5)	40	52.7 (10.5)	129	44.3 (11.0)	109	49.1 (10.5)
Times sang song, P	49	1.8 (1.0)	33	2.4 (0.8)	137	1.5 (0.82)	120	2.0 (0.84)
Times sang song, NP	51	1.7 (1.0)	33	2.5 (0.8)	137	1.6 (0.83)	116	2.1 (0.82)
Object memory test								
Utilization <sup>b</sup>	54	1.3 (3.1)	38	0.6 (1.8)	149	0.4 (1.2)	120	0.4 (1.6)
Immediate recall (0–18)	54	7.7 (2.0)	38	8.2 (2.7)	149	7.2 (2.3)	120	8.7 (2.3)
Recognition (0–32)	54	31.4 (2.0)	37	31.6 (1.4)	143	31.4 (1.7)	118	31.8 (0.4)
Purdue pegboard test (0–25) <sup>c</sup>								
Pegs, P	54	13.1 (2.2)	38	13.8 (1.9)	150	13.0 (2.3)	124	14.0 (2.1)
Pegs, NP	54	11.4 (2.3)	38	12.7 (1.8)	149	11.6 (2.4)	123	12.6 (2.1)
Pegs, both	54	9.5 (2.4)	38	10.4 (2.0)	148	9.8 (2.2)	123	10.5 (2.2)
Visual motor integration test								
Figure copying score	53	19.5 (4.2)	42	21.0 (4.1)	138	19.4 (3.7)	118	20.4 (3.6)
Name writing test								
P Hand, s <sup>b</sup>	53	9.1 (6.4)	38	7.3 (4.7)	143	10.9 (8.6)	121	9.5 (8.4)
NP Hand, s <sup>b</sup>	53	20.7 (11.3)	38	18.6 (10.9)	140	22.0 (10.7)	118	19.9 (11)

Abbreviations: SD, standard deviation; P, preferred hand; NP, non-preferred hand; ms, milliseconds; s, seconds.

<sup>a</sup> N = total children that completed all the neurobehavioral tests.

<sup>b</sup> Lower score indicates better performance.

<sup>c</sup> Average scores of trial 1 and trial 2 for each Purdue pegboard test outcome.

**Table 5**  
Linear regression models of neurobehavioral tests and parent's occupation (agricultural children vs. non-agricultural) at two time points and change over time, negative coefficients [ $\beta$ ] indicate that performance is worse with increasing exposure.

NB Outcome <sup>c</sup>	Agricultural children vs. non-agricultural children					
	First visit <sup>a,d</sup>		Second visit <sup>a,d</sup>		Difference in NB performance <sup>e</sup>	
	N	$\beta$ (SE)	N	$\beta$ (SE)	N	$\beta$ (SE)
Digit span						
Forward	182	<b>-0.34 (0.15)</b>	151	0.16 (0.18)	125	0.26 (0.19)
Reverse	139	0.04 (0.14)	127	0.06 (0.18)	92	0.09 (0.23)
Finger tapping						
Taps, P	187	2.27 (1.40)	152	0.95 (1.59)	128	-1.67 (1.68)
Taps, NP	187	2.6 (1.38)	151	1.25 (1.61)	127	-1.73 (1.56)
Match-to-sample						
Number correct	176	0.30 (0.33)	151	-0.42 (0.32)	122	-0.27 (0.43)
Latency	176	-79.43 (148.79)	151	-17.47 (204.19)	122	57.47 (264.97)
Symbol-digit						
Latency	180	132.72 (197.19)	146	366.22 (227.36)	121	27.54 (214.00)
Continuous performance						
Correct Hits	182	0.02 (0.02)	146	0.02 (0.02)	122	0.01 (0.03)
d prime	175	0.24 (0.15)	146	0.27 (0.16)	122	0.16 (0.22)
Divided attention						
Taps, P	177	2.33 (1.33)	146	-1.05 (1.53)	117	<b>-4.13 (1.92)</b>
Taps, NP	177	1.45 (1.32)	145	0.79 (1.36)	116	-1.03 (1.49)
Taps with song, P	177	1.71 (1.59)	146	-0.15 (1.53)	117	<b>-3.89 (1.93)</b>
Taps with song, NP	177	1.70 (1.30)	145	0.26 (1.39)	116	-1.77 (1.45)
Times sang song, P taps	181	<b>-0.24 (0.12)</b>	150	<b>-0.37 (0.14)</b>	119	-0.29 (0.16)
Times sang song, NP taps	183	-0.15 (0.12)	146	<b>-0.48 (0.14)<sup>b</sup></b>	121	<b>-0.36 (0.14)</b>
Object memory						
Utilization	198	<b>0.65 (0.30)</b>	155	0.10 (0.29)	134	-0.70 (0.46)
Immediate recall	198	-0.38 (0.29)	155	0.58 (0.39)	134	0.77 (0.47)
Recognition	192	-0.17 (0.29)	152	-0.04 (0.14)	127	0.45 (0.26)
Purdue pegboard						
Pegs, P	198	0.06 (0.23)	158	0.13 (0.28)	136	-0.25 (0.32)
Pegs, NP	197	0.23 (0.24)	157	-0.27 (0.26)	135	<b>-0.66 (0.31)</b>
Pegs, both	196	0.36 (0.25)	157	0.07 (0.33)	135	-0.50 (0.38)
Visual motor integration						
VMI score	188	-0.23 (0.43)	160	-0.83 (0.55)	131	-0.63 (0.62)
Signature test						
P Hand	190	-1.36 (1.24)	155	-1.94 (1.37)	130	1.23 (1.27)
NP Hand	187	-0.73 (1.76)	152	-1.23 (1.93)	127	1.38 (2.71)

Abbreviations: NB, neurobehavioral;  $\beta$ , regression parameter estimates; SE, standard errors; P, preferred hand; NP, non-preferred hand. Significance ( $p < 0.05$ ) marked in bold numbers and grey cell background.

<sup>a</sup> Models represent the effect of agricultural children vs. non-agricultural children (referent group) on continuous neurobehavioral outcomes [ $\beta$ ].

<sup>b</sup> Significant after Bonferroni corrections ( $p < 0.0021$ ) with a  $p$ -value = 0.001.

<sup>c</sup> All outcomes have been standardized so that a negative coefficient indicates that performance is worse with increasing exposure.

<sup>d</sup> Parent's occupation Time 1 and Time 2 models adjusted for age, gender, and CBCL external score.

<sup>e</sup> Longitudinal parent's occupation models adjusted for HOME score and mother's education. Additional test-specific confounders include: gender (name writing tests), and computer use (name writing tests and object memory tests).

dust samples at tenfold to hundredfold times higher than levels reported in the current study. In 1999, Rothlein et al. found median levels of 4400 ng/g of phosmet, 5300 ng/g of azinphos-methyl, 180 ng/g of malathion and 130 ng/g of chlorpyrifos [39]. In 2001, McCauley et al. found median levels of azinphos-methyl at 1450 ng/g in farmworkers homes [14]. The lower levels reported in the current study are likely due to regulation changes during this time period by the U.S. Environmental Protection Agency (EPA). During 2000–2001, the residential use of chlorpyrifos was banned [40] and new restrictions were placed on the agricultural use of phosmet and azinphos-methyl [41,42]. Interestingly, a large increase in malathion residues was observed in the homes of agricultural families in 2011. It is hypothesized that this was likely due to a spotted wing drosophila infestation in the orchards in the study region during this time [2010–2011] and malathion was recommended as a choice of protective sprays during this period [43].

#### 4.2. Associations between exposure measures and neurobehavioral performance

It has been hypothesized that practice effects, which reflect learning potential, could help detect mild cognitive impairment [44,45]. A study conducted by Nguyen et al. (2015) examined

cognitive function and learning capacity between adult Latino farmworkers and Latino non-farmworkers at baseline and a 3-month follow-up [46]. Although the performance of both groups improved from baseline to testing at 3-months, non-farmworkers showed improved performance on more of the outcome measures than the farmworker populations. The authors observed that these improvements or practice effects indicate the learning potential of the participants. Although performance was similar at baseline, the farmworkers demonstrated deficits in learning potential.

In this study, practice effects were also observed among both the agricultural and non-agricultural children, both groups improved on all the neurobehavioral tests at Time 2 compared to Time 1, with the exception of the latency scores on the Match-to-Sample test for the non-agricultural group. These learning effects are expected since children between the ages of 5–12 are experiencing rapid development. However, the two groups appeared to have differences in practice effects, i.e., learning potential. An examination of the overall data revealed a pattern of deficits in learning among children whose parents work in agriculture; these children showed less improvement on outcome measures compared to children from non-agricultural families. Agricultural children showed less improvement on tests assessing motor function (i.e., Finger Tapping, Divided Attention (including

tapping with and without distraction), Purdue Pegboard, and Visual Motor Integration) and memory (i.e., Object Memory).

Motor function and memory deficits have been reported in other studies examining pesticide exposure in children. Preschool children from agricultural families performed worse on Finger Tapping and Match-to-Sample tests compared to non-agricultural children from a similar community [8]. Preschool children in Mexico had deficits in eye-hand coordination and memory [47]. Adolescent pesticide applicators performed significantly worse than the controls on neurobehavioral tests measuring memory and attention [6].

Although, we see deficits in learning from Time 1 to Time 2, there were few significant differences between the two groups at Time 1 and Time 2 and only one association was significant after applying Bonferroni corrections. Several factors may account for the lack of associations. Even though we controlled for age, the range of the ages of the children is quite broad and cognitive abilities of a 5-year-old are very different than a 12-year-old. Perhaps we did not follow the children long enough to identify neurobehavioral deficits in performance, deficits can be subtle and may be difficult to detect, especially if the exposure is at a low level, and some cognitive processes may not be affected until a threshold concentration within the nervous system is exceeded. Following the children for a longer period of time would provide more information about the developmental changes over time. Also there may be less exposure for this age group since school-age children have less hand-to-mouth activity and are usually on the floor less than younger children. Finally, both agricultural and non-agricultural homes had detectable pesticide residues in which we would infer that non-agricultural children had some OP exposures.

No clear patterns between the two exposure measures emerged and this might be due to the timing of exposures. The time of exposure to OP pesticides is important to assess since there are critical stages in the development of a child's nervous system, current studies indicate that prenatal exposure may have a negative effect on a child's neurodevelopment [11,12]. The OP residues in the house dust measures potential current exposures and parent's occupation is potentially measuring accumulated exposures (current and past exposures, even exposures prior to the child's birth, while the mother was pregnant). Questionnaire data showed that 117 agricultural children (75%) had either their mother and/or father working in agriculture throughout their whole lives, including during pregnancy. This might explain why more effects are observed with parent's occupation compared to OP dust measures. The effects of past pesticide exposures may be lasting and may differ from the effects of current exposures.

Additional limitations include the convenience sample of the participants which may not be representative of the population and potential misclassification with parent's occupation. To check for any misclassifications, we examined other agricultural-related questions to verify parent' occupations. Furthermore parent's occupation and pesticide dust residues provide only a measure of potential exposure and there is no information on actual exposures. Another limitation is the large number of models analyzed, caution should be taken on the few significant results observed in the models. When the *p*-value level was adjusted according to the respective number of comparisons (Bonferroni correction) to  $p \leq 0.002$ , this resulted in only one significant association, parent's occupation at Time 2 with the Divided Attention test (times sang song while tapping with non-preferred hand). There may be other cultural factors unaccounted for between the groups that may impact performance. With the dust samples, there was a large proportion of imputed values for azinphos-methyl which has the potential to create a bias. The use of OP molar total assumes a common receptor pathway and a similar metabolic profile for these four OPs. Carbaryl, a carbamate

insecticide, had also been used in the orchards during the study period, and exerts a similar mechanism of action by inhibiting the acetylcholinesterase enzyme. There is a possibility that carbamate exposures contributed to the neurobehavioral effects presented in this study.

However, in spite of these limitations this present study has several strengths. The longitudinal design allowed us to observe changes in exposure and neurobehavioral performance over time, many cross sectional studies typically only measure exposure and performance at a single time point. We also were able to observe practice effects, i.e., learning potential, of the children with repeated tests. Identifying differences in learning potential could help detect mild cognitive impairment [46]. We thoroughly assessed potential confounders, adjusting for several demographic and behavioral indicators. Age, gender, and mother's education have been known to impact neurobehavioral performance. We also controlled for CBCL external scores, acculturation, home environment, mother's education, and home computer use, which could impact performance on the neurobehavioral test battery. Another strength is that the children included in this analysis had similar socio-economic backgrounds.

### 4.3. Conclusions

This study demonstrates that children living in an agricultural community are exposed to OPs. In spite of the EPA phase-out of OPs, both agricultural and non-agricultural homes contained OP residues. Higher pesticide residues were found in homes of children with parents were working in agriculture. Furthermore, higher levels of malathion, five times higher than the previous years, were found in 2011. Integrated pest management protocols specify that this pesticide was the recommend method for controlling the spotted wing drosophila infestation [43]. This indicates that agricultural pesticide use may contribute to exposures in agricultural and non-agricultural children and historical residential use may also contribute to these exposures.

Despite these lower OP levels, there is still concern about the impact on the development of children. Agricultural children had more OPs in their homes, at higher amounts, and showed deficits in learning on neurobehavioral performance compared to non-agricultural children. These findings suggest that OP exposures may cause deficits in learning over time in agricultural communities where children are at an increased risk from pesticides. This study provides additional support that low level OP exposures might be associated with neurobehavioral performance in children. More research is needed to further define the nature of these deficits in agricultural communities.

More broadly, our findings imply that there is a need to educate agricultural communities on the potential health impacts of agricultural pesticide use and efforts should be made to promote effective strategies to reduce exposures in the household.

### Competing financial interests

Oregon Health and Science University (OHSU) and Dr. Rohlman have a significant financial interest in Northwest Education Training and Assessment, LLC, a company that may have a commercial interest in the results of this research and technology. This potential conflict of interest was reviewed and a management plan approved by the University of Iowa and the OHSU Conflict of Interest in Research Committee was implemented.

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