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NEUROBEHAVIORAL EFFECTS OF CHRONIC LOW-LEVEL PESTICIDE EXPOSURE IN CHILDREN

by

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FINAL THESIS

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Department of Public Health and Preventive Medicine

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CERTIFICATE OF APPROVAL

This is to certify that the Master's thesis of

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has been approved

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Table of Contents **Page**

List of Tables **Page**

List of Figures Page

List of Appendices

Abbreviations

AG	agriculture
BARS	Behavioral Assessment and Research System
CBPR	Community-Based Participatory Research
DAP	dialkylphosphate
DEP	diethylphosphate
DETP	diethylthiophosphate
DMDTP	dimethyldithiophosphate
DMP	dimethylphosphate
DMTP	dimethylthiophosphate
LOD	Limit of Detection
NHANES	National Health and Nutrition Examination Survey
Non-AG	non-agriculture
OP	organophosphate
PENTB	Pediatric Environmental Test Battery
SD	standard deviation
μg/g	microgram per gram

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I would like to thank my thesis committee, Dr. William Lambert, Dr. Diane Rohlman, and Michael Lasarev for their support, interest and commitment to this research topic. It’s been a pleasure working with each one of my committee members.

Dedication

I would like to dedicate this thesis work to my father, Harry J. Rectenwald, a fellow scientist who has taught me to value the health of our environment and inspired me to pursue a career in Public Health.

Abstract

Studies of children living in agricultural communities have identified potential health risks associated with chronic environmental exposure to pesticides, including neurologic impairment. Young children have a greater vulnerability to effects of pesticide exposure than adults because sensitive organ systems are in the process of developing and their capacity to eliminate toxins is not mature. This secondary analysis using data collected in a community-based participatory research program conducted between 1996 and 2006 combines data collected on children of Latino farm workers living in agricultural areas of Oregon. This analysis demonstrates that children of agriculture workers are more likely to be exposed to more organophosphate pesticides than children of non-agriculture workers. The highest concentrations of individual dialkylphosphate (DAP) metabolites in urine samples were detected at the middle of the growing season when exposure to pesticide residues was expected to be high. Children of non-agriculture workers with urine samples containing more than one type of DAP metabolite performed better than children of agriculture workers on neurobehavioral measures related to attention. These observations were made across communities hosting varying agricultural industries and types of crops. Neurobehavioral effects are just one negative health effects of pesticide exposure; there are many other health effects currently under investigation and possibly not yet considered. Precaution should be taken to protect the health of the families working in agriculture within the United States and around the world.

Chapter I: Introduction

Background

The neurobehavioral effects of acute pesticide exposure have been well documented; however, relatively few studies have examined the effect of low-level exposure. Animal models have demonstrated harmful neurologic effects of low-level chronic pesticide exposure (Moser, 2007), and epidemiological studies of occupational exposure to pesticides have demonstrated deficits on several neurobehavioral tests or measures (Stephens et al. 1995; Bazylewicz-Walczak et al., 1999; Farahat et al., 2003). In light of the growing evidence showing harmful neurologic effects of organophosphate (OP) pesticides in adults, concerned scientists have questioned whether children are at risk of similar neurologic effects.

Young children are more vulnerable to effects of pesticide exposure than adults, because sensitive organ systems are in the process of developing and their capacity to eliminate toxins is not mature (Faustman et al., 2000). Like adults, children are exposed to pesticides through multiple pathways including food, drinking water and residential pesticide use. Children of agriculture workers may be exposed to more pesticides through pathways related to the proximity of homes to fields where pesticides are applied, and carry-home transport of pesticide residues on boots, clothing, and skin (Fenske et al., 2000; Lu et al. 2000; McCauley et al., 2001).

Studies of children living in agricultural communities have identified an increased risk of impairment associated with chronic low-level pesticide exposure (Guillette et al., 1998; Rohlman et al. 2005; Handal et al., 2007). In Oregon, Latino families working in agriculture have been the focus of a community-based participatory research (CBPR) project conducted between the years 1996 and 2006. The major goal of this CBPR project was to better understand how the agriculture work environment, including exposure to pesticides, affects the health of this high-risk population of Latino farm workers and their families (McCauley, 2001).

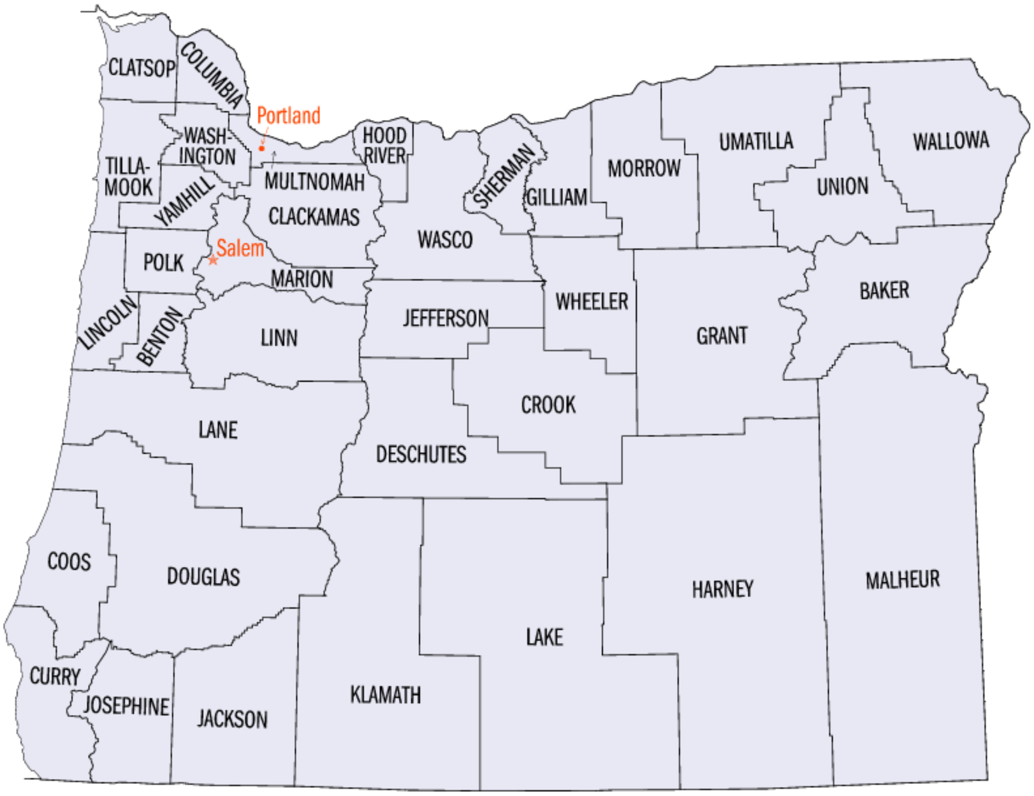

Figure 1-1. Oregon Counties

<u>Exposure: OP Pesticides,</u>

Recognizing that there are worldwide public health benefits of pesticide use, this analysis will explore potential adverse effects of pesticide exposure. Benefits of pesticide use include the treatment of drinking water and the control of disease vectors; pesticides have lead to increased production of food, management of invasive species and beautiful gardens. Nonetheless, pesticides are inherently toxic and function by harming, destroying, or repelling living organisms, including human beings. Some pesticides are broad-spectrum biocides and others target specific organisms. Examples include herbicides, insecticides, rodenticides, fungicides, fumigants and wood treatment products. Low-levels of pesticides are present in our food, water, air, and land.

Unfortunately, many pesticides currently registered in the United States (US) have not been tested for toxic effects on human or ecological health (Faustman et al., 2000) and the public health consequences of chronic exposure to low-levels of pesticides are not well understood. This analysis will focus on a class of pesticides, called organophosphate (OP) pesticides. OP pesticides are the most heavily used insecticide in the US. Annually, approximately 73 million pounds of OP pesticides are used in the US, this accounts for 70% of insecticide use in the US (USEPA, 2001).

OP pesticides function by interfering with the nervous system of insects and other living organisms inadvertently exposed, including humans. OP pesticides inhibit cholinesterase, an enzyme that breaks down acetylcholine. Acetlycholine is a neurotransmitter that allows the nerves to function properly. Inhibition of cholinesterase by OP pesticides leads to the accumulation of acetylcholine interfering with proper nerve function, resulting in continued stimulation and then suppression of neurotransmission (Slotkin et al. 2006). Symptoms of acute exposure include nausea, vomiting, cholinergic effects, weakness, paralysis and seizures. The effects of acute high-level exposure to OP pesticides are known to cause neurologic dysfunction; however, the effects of chronic low-dose exposure to OP pesticides are not well known.

In Oregon, as in other parts of the country, OP pesticide exposure is not restricted to agricultural areas. Within non-agriculture communities, pesticide exposure typically occurs through multiple pathways, including food, drinking water, and residential pesticide use. These same pathways are present in agriculture communities, alongside additional pathways related to the proximity of homes relative to fields where pesticides are applied, and carry-home transport of residues on boots, clothing, and skin (Fenske et al., 2000; Lu et al. 2000; McCauley et al., 2001). Within agriculture communities, pesticide exposure may vary. For example factors affecting inter-community variability may include: crop type (Lambert et al., 2006) and growing season (Berkowitz et al., 2003; Whyatt et al., 2003). OP pesticides applied on crops in Oregon may include one or more of the compounds listed in Table 1-1. OP pesticides are not the only type of pesticide applied to crops in Oregon, other types of pesticides and fertilizers may be applied to crops in addition to or instead of OP pesticides.

Pesticide exposure varies by individual. Age is one factor affecting individual pesticide exposure. Children ages 0-6 are more likely to have higher exposure to pesticides because children may come in direct contact with pesticide residues on parent's work clothing (Lu et al., 2000); children crawl on the floor (possibly eat what they find on the floor) and engage in less hand washing and more hand-to-mouth activity than older school age children (Freeman et al., 2001). School-age children (ages 7-11) likely have lower exposure to pesticides than younger children. Children in this older age group generally spend more time outside and away from the home and orchards; take the bus home rather than the family cars where work clothes and boots have been; spend less time on the floor and are less likely to eat what they find on the floor; school-age children engage in more hand washing and less hand-to-mouth activity (Freeman et al., 2001). Gender differences in behavior that may also affect pesticide exposure (Freeman et al., 2001).

OP pesticide exposure can occur via ingestion, inhalation, dermal absorption or ocular contact. There is significant variation in the relative absorption by these various routes. For example, the lethal dose for 50% of rats tested (LD_{50}) for oral ingestion of parathion ranges between 3 and 8 mg/kg; while, the LD_{50} for dermal adsorption is 8 mg/kg. Absorption of OP pesticides also varies by the individual exposed (Reigart et al., 1999). The breakdown of OP pesticides occurs predominantly by hydrolysis in the liver and rates of hydrolysis vary between children (intra child variability) and within a child (inter child variability) (Adgate et al., 2001).

Biomarkers of exposure are indictors of internal dose, or the amount of chemical exposure that has resulted in adsorption into the body. Dialkylphosphate (DAP) metabolites are biomarkers of organophosphate exposure. About 75% of registered OP pesticides are metabolized into measureable DAP metabolites in urine samples (CDC, 2005). Measurements of these metabolites in urine reflect recent exposure occurring via multiple pathways within the past few days. Table 1-1 presents the type of urinary DAP metabolite and its parent OP compound. This study will include measurement of DAP levels in urine for the following 5 metabolites:

- dimethylphosphate (DMP)
- dimethylthiophosphate (DMTP)
- dimethyldithiophosphate (DMDTP)
- diethylphosphate (DEP)
- diethylthiophosphate (DETP)

Detectable concentrations of urinary DAPs are not necessarily indicative of an adverse health outcome. This analysis will examine the relationship between urinary DAPs and neurobehavioral test performance. The relationship between urinary DAPs and acetylcholinesterase enzyme activity has not been well defined in the literature.

Table 1-1. Organophosphate pesticides and their metabolites (CDC, 2005)

Pesticide	DMP	DMTP	DMDTP	DEP	DETP
Azinphos methyl	●	●	●		
Chlorethoxyphos				●	●
Chlorpyrifos				●	●
Chlorpyrifos methyl	●	●			
Coumaphos				●	●
Dichlorvos (DDVP)	●				
Diazinon				●	●
Dicrotophos	●				
Dimethoate	●	●	●		
Disulfoton				●	●
Ethion				●	●
Fenitrothion	●	●			
Fenthion	●	●			
Isazaphos-methyl	●	●			
Malathion	●	●	●		
Methidathion	●	●	●		
Methyl parathion	●	●			
Naled	●				
Oxydemeton-methyl	●	●			
Parathion				●	●
Phorate				●	●
Phosmet	●	●	●		
Pirimiphos-methyl	●	●			
Sulfotepp				●	●
Temephos	●	●			
Terbufos				●	●
Tetrachlorvinphos	●				

Notes: Shaded indicates OP pesticides utilized in at least one agricultural community in this analysis

Outcome: Neurobehavioral Test Performance

Neurobehavioral tests are an assessment of the condition of an individual's nervous system through an observed behavior. Psychologists have developed various test batteries to measure the effects of toxic exposures on the nervous system. Because the impact of OP pesticides on NB functioning is unknown, a wide range of neurobehavioral functions is included in this analysis. Performance on neurobehavioral tests is impacted by complex interactions among genetic, environmental and social factors that may affect children during vulnerable periods of development. Table 1-2 presents a summary of the neurobehavioral tests used in this analysis.

Table 1-2. Summary of neurobehavioral tests included in this analysis

Name of Test	Function and Description
BARS Digit Span	Memory and Attention - Spoken presentation of number sequences - Two chances at each span length
BARS Finger Tapping	Response Speed and Coordination - Right and left hand tested - Number of taps in 20 second duration
BARS Match-to-Sample	Visual Memory - 15 stimuli shown for 3 seconds - Choose from three choices - Delay between presentation and choice varies from 1 to 8 seconds
BARS Continuous Performance	Attention - 75 shapes shown rapidly, 30 targets - Press key when target (circle) was shown
BARS Divided Attention	Divided Attention - Tap button while reciting nursery rhyme - Right and left hand tested
Object Memory Test	Recall and Recognition Memory - Shown 16 objects and asked to name - Immediate and delayed recall; recognition test
Purdue Pegboard	Dexterity - Number of small pegs placed in holes during 30 seconds - Right, left and both hand trials
Visual Motor Integration	Hand-eye coordination - Copied line drawings

Rohlman et al. (2000) assembled the test battery used in this analysis. This test battery combines the following: computerized tests from the Behavioral Assessment and Research System (BARS); the Purdue Pegboard and Visual Motor Integration tests, two performance tests adapted from the Pediatric Environmental Test Battery (PENTB); and a test of recall and recognition, the Object Memory Test. Performance tests from the BARS have been used in several studies examining pesticide exposure in children and adolescents (Rohlman et al., 2001a, 2005, 2007a).

Demographic variables are known to impact performance on neurobehavioral tests in children and adults (Rohlman et al., 2007a). Other factors influencing neurobehavioral test performance include: exposure to other toxicants, prenatal influences, nutrition, genetic predisposition, and socio-environmental influences over the lifetime of a child (Dietrich and Bellinger, 1994; Jacobson and Jacobson, 2005; Weiss and Bellinger, 2006). This thesis analysis will explore the potential effect of OP pesticide exposure on neurobehavioral test performance, alongside other factors that may lead to differences in neurobehavioral test performance.

Review of the Literature

Animal studies have demonstrated that OP insecticides affect mammalian brain development (Casida et al., 2004). Specifically, long-term exposure (>30-days) to OP pesticides adversely affects neurologic development and function. In general, animal studies have reported cognitive changes, altered neuromotor/sensory function, impaired vestibular function, and electrophysiological changes (Moser, 2007). Findings from animal studies can provide insight into expected hazards in human populations. However, experimental differences between animal and human studies such as dosage, duration of exposure, route of exposure, specific endpoints measured, and the controlled laboratory environment of animal studies, must be taken into consideration.

Epidemiological studies examining the relationship between long-term pesticide exposure and neurologic health have reported neurobehavioral changes in adults exposed to pesticides in the workplace. Such studies found deficits in the following neurobehavioral measures: sustained attention, information processing, motor speed and coordination (Stephens et al., 1995; Bazylewics-Walczak et al., 1999; Fiedler et al., 1997; Kamel et al., 2003).

Until recently, relatively few studies have examined chronic environmental exposure and NB outcomes in children. Jurewicz et al. (2008), recently conducted a review of eight epidemiological studies assessing neurobehavioral effects of prenatal and childhood exposure to OP pesticides. Six of the studies reviewed were performed in the US, and two were performed outside of the US. Study participants ranged in ages from newborns to adolescents. Although methods for classifying the exposure and outcomes varied in each of the eight studies, all revealed that exposure to OP pesticides was associated with neurodevelopmental disorders (Table 1-3).

Epidemiological studies outside the US are of particular interest because environmental exposure to pesticides in developing countries can be relatively high in comparison to the US. Handal et al. (2007) conducted a study in children (ages 3-61 months) in Ecuador. Results demonstrated deficits on gross and fine motor and socio-individual skills. Guilette et al. (1998) conducted a study in children (ages 5-6 years) in Mexico and demonstrated deficits in coordination, stamina, memory and figure drawing.

Farm worker families in the US are potentially exposed to more pesticides than families not working in agriculture. In Oregon, the CBPR Project of McCauley and colleagues (2001) identified potential neurologic impairment associated with chronic environmental exposure to pesticides. Rohlman et al. (2005) identified potential health risks, including neurologic impairment, associated with chronic environmental exposure to pesticides. Specifically, neurobehavioral test results demonstrated modest differences in measures of response speed (Finger Tapping) and latency (Match-to-Sample) in AG children compared to Non-AG. Rohlman et al. (2005) compared Non-AG and AG children, but did not include an objective measure for pesticide exposure, such as urine samples.

Table 1-3. Summary of studies examining the relationship between chronic OP exposure and neurobehavioral outcomes in children (Jurewicz et al., 2008)

Study Reference	Study Population	Exposure Measure	Outcome Measure	Significant Finding
Eskanzai et al. (2007)	Mexican-American children at 6 months (N=396) and 24 months (N=372)	Maternal and child urinary DAP concentration	Bayley Scales of Infant Development [Mental Development (MDI) and Psychomotor Development (PDI) Indices] and mother's report on the Child Behavior Checklist (CBCL)	Adverse associations of prenatal DAPs with mental development and pervasive development problems at 24 months of age. Results should be interpreted with caution given the observed positive relationship with postnatal DAPs.
Young et al. (2005)	US Latino infants </= 2 months (N = 381)	Prenatal and postnatal urinary DAP concentration	7 clusters on the Brazelton Neonatal Behavioral Assessment Scale (BNBAS)	For infants >3 days old, increasing average prenatal DAP concentrations were associated with an increase in both the number of abnormal reflexes and the proportion of infants with more than three abnormal reflexes (Total DAP adjusted OR=4.9, 95% I = 1.5, 16.1). No detrimental associations were found between average postnatal DAP concentrations and BNBAS clusters.
Ruckart et al. (2004)	US children exposed (N = 251) and unexposed (N=401) to methyl parathion	Children urinary and environmental para-nitrophenol concentrations	Pediatric Environmental Neurobehavioral Test Battery (PENTB)	Exposed children had more difficulty performing tasks that involved short-term memory and attention. There were no differences in test scores for general intelligence and integration of visual motor skills.
Rauh et al. (2006)	US children 12 months, 24 months and 36 months (N=254)	Umbilical cord plasma analyzed for chloropyrifos	Bayley PDI and MDI; CBCL	The adjusted mean 36-month PDI and the MDI scores of the highly and lower exposed groups differed by only 7.1 and 3.0 points, respectively. However, the proportion of delayed children in the high-exposure group, compared with the low-exposure group was 5 times greater for the PDI, and 2.4 times greater for the MDI.
Grandjean et al. (2006)	Ecuadorian school aged children (N=79)	Maternal occupation during pregnancy, child DAP concentration	Simple reaction time, Santa Ana dexterity test, Stanford-Binet copying, and Wechsler Intelligence Scale for Children-Revised Digit Spans forward	The Stanford-Binet copying test showed a lower drawing score for copying designs in exposed children than in controls. Increased DAP concentrations were associated with increased reaction time and no other outcome.
Engel et al. (2007)	US neonates before hospital discharge (N=311)	Maternal Urine samples analyzed for DAPs	Brazelton Neonatal Behavioral Assessment Scale	Higher levels of total dimethylphosphates and total DAPs were associated with an increase in abnormal reflexes.
Rohlman et al. (2005)	US children age 48-71 months (N=78)	Parental occupation (AG, or Non-AG)	5 tests from Computerized BARS and 3 non-computerized tests: Object Memory, Purdue Pegboard, and VMI	Male children of agricultural workers performed significantly worse than male children from non-agricultural workers on right hand Finger Tapping and latency measures (Match-to-Sample test).

Rationale for Thesis Research

Epidemiological studies investigating pesticide exposure and neurobehavioral effects in children will help us to understand and support safe use of pesticides. Pesticides are ubiquitous in our environment and although current environmental regulations aim to protect children from harmful environmental contaminants, the safety of pesticides has not been tested. OP pesticides are neurotoxins that may lead to neurological impairment in children, which is a public health concern with far reaching social implications.

This is a secondary analysis of urine samples and neurobehavioral test results collected during the Oregon CBPR Project (McCauley et al., 2001). This analysis will combine data collected in 2002 and 2004 and includes samples collected at three time points in 5 different counties. Study participants were children of Latino farm workers; a subpopulation of children in the US potentially exposed to more pesticides and therefore, experiencing a greater risk of potential health effects.

Results of this analysis will be used to support the growing body of literature on the effects of OP pesticide exposure on the nervous system, to inform risk assessment of pesticide exposure in humans and, most importantly, to identify the need for public health action within the US and internationally.

Specific Aims

This thesis assesses the exposure-response relationship between OP pesticides and neurobehavioral test performance. The specific aims are to:

1. Select a representative measure of OP exposure by assessing the seasonal variation of individual DAP compounds detected in urine samples at the beginning, middle and end of the growing season.
2. Test the association between pesticide exposure and agriculture status after adjusting for potential confounding factors (including age, gender, and maternal education).
3. Test the association between pesticide exposure and neurobehavioral performance in children of agricultural workers and non-agricultural workers, after adjusting for potential confounding factors (including age, gender and maternal education).

Main Hypothesis

Children of agricultural workers are exposed to more pesticides and will perform worse on neurobehavioral tests, when compared to children of non-agricultural workers.

Chapter 2: Methods

Demographics

This is a secondary analysis of urine samples and neurobehavioral test scores collected from children residing in five counties in Oregon. The occupation of the parent, non-agricultural (Non-AG) versus agricultural (AG), is the main predictor of interest in this analysis. All covariates were assessed for AG and Non-AG children using appropriate summary measures in order to identify differences between the two groups. A standard two-sample *t*-test was used to assess statistically significant differences between means and a test of two proportions (*z*-test) was used to assess statistically significant differences between the two groups (2-sided $p<0.05$).

Urine Analysis

Urine samples were collected from each study participant and collection coincided with neurobehavioral test administration. Convenience samples were collected mid-morning through early afternoon using commode inserts. Sample analysis was completed by the Center for Research on Occupational and Environmental Toxicology laboratory in Portland, Oregon.

Each sample was analyzed for five DAPs (DEP, DMTP, DETP, and DMDTP) by gas chromatography according to a modified method of Moate et al. (1999). Aliquots of the samples underwent azeotropic distillation with methanol and evaporation under a nitrogen stream. Sample extracts were then derivatized with 2,3,4,5,6-pentalfluorobenzylbromide to convert phosphate acids to esters. Extracted samples were analyzed on a gas chromatograph (model 5890; Hewlett-Packard, Pal Alton, CA) equipped with a pulsed flame photometric detector (OI Analytical, College Station, TX). Table 2-1 presents the limits of detection (LOD) for each of the five metabolites.

Table 2-1. Limits of Detection (LOD)

OP Metabolite	LOD (ng/mL)
DMP	4.0
DEP	2.0
DMTP	2.2
DMDTP	1.6
DETP	1.6

Creatinine levels were also measured in all urine samples included in this analysis. Creatinine concentrations were determined by the modified Jaffe rate method (Sigma Diagnostics Creatinine Kit no 555; Sigma Aldrich, St. Louis, MO). Additional details on urine sample collection and analytical methods are described in Lambert et al. (2005).

Statistical Analysis of Urine Samples

Pesticide Exposure Over Time

Urine samples were collected at 3 time points: the beginning (Time 1), middle (Time 2) and end (Time 3) of the growing season. Table 2-2 presents the dates of sample collection for each county. Note that urine samples were not collected at Time 3 in Jackson County. Available samples at all 3 time points were analyzed to assess variation in pesticide exposure across the growing season. Measures of pesticide exposure included both the number of detected DAPs and the individual DAP concentrations. The number of detected DAP metabolites and individual DAP concentrations provide a measure of acute pesticide exposure and parental occupational status provides a measure of chronic pesticide exposure.

Table 2-2. Sample Collection Dates

	Time 1	Time 2	Time 3
Hood River	August 2002	September 2002	October 2002
Jackson	June 2004	July 2004	NA
Lincoln	March 2002	April 2002	May 2002
Multnomah	June 2002	July 2002	August 2002
Washington	June 2002	July 2002	August 2002

NA = not applicable because samples not collected

The number of DAPs detected was counted for each child at each time point. A chi-square test was conducted to assess whether or not the change in the proportion of DAPs detected was different between Non-AG and AG children for Time 1 compared to Time 2, Time 2 compared to Time 3, and Time 1 compared to Time 3.

Prior to summarizing DAP concentrations over the growing season; the distribution of creatinine was examined at each time point. Urine samples less than the 5th percentile and greater than the 95th percentile were excluded from further analysis because of concerns of hydration state and metabolic disorders (Lowenherz et al. 1997, Lu et al. 2001). All sample results were adjusted for the level of creatinine.

Individual DAP concentrations were summarized using the geometric mean. The geometric mean is a descriptive statistic to summarize the data and is an estimator the population median. Geometric mean concentrations were calculated utilizing half of the LODs for non-detected DAP metabolites. The proportions of non-detected concentrations were calculated for each DAP, in order to assess how non-detected results influenced the geometric mean. The decision to include half of the LODs was based on the fact that LODs were relatively high in this study and lead to a higher proportion of non-detected values. The comparison of geometric mean DAP concentrations identified general trends and are not necessarily representative of true population concentrations. An ANOVA was conducted to determine if the change in the geometric mean of individual DAPs at each of the time points was different between Non-AG and AG children (2-sided $p<0.10$). Because the geometric mean was used to summarize data, analyses were completed on the logarithmic scale and exponentiation was used to return the computation to the original value.

Pesticide Exposure at Time 2

Based on the results of the temporal analysis, urine samples from Time 2 (mid-point) were selected to represent the peak level of exposure in the harvest season. A multivariable logistic regression model was applied to determine if AG children have a greater proportion of detectable pesticide metabolites than Non-AG children, after adjusting for age, gender, maternal education, and county of residence. A likelihood ratio test was used to test potential interactions for agriculture status with age and mother's education (2-sided $p<0.10$), and standard Wald tests were used to assess individual effects.

Neurobehavioral Analysis

Neurobehavioral tests were administered individually to each child. The child was able to choose the language he/she felt most comfortable taking the test. The children completed the neurobehavioral battery up to three times that coincided with urine sample collection (Table 2-2). The sessions occurred either in a mobile testing vehicle or in a room at the Head Start or community center. The neurobehavioral battery consisted of five tests from the computerized Behavioral Assessment and Research System (BARS) and three non-computerized tests, Object Memory, Purdue Pegboard, and Visual Motor Integration. The computerized tests are administered using a durable response unit with nine buttons that is placed over a keyboard to minimize the impact of working on a potentially intimidating device such as a computer keyboard (Rohlman et al., 2003). During each test session a trained examiner was present to read instructions, answer questions and reinforce c found in Rohlman et al. (2000, 20001a, 2001b, 2003).

Data from the second time period was selected for this analysis to coincide with the timing of the measure of pesticide exposure using urine biomarkers. Use of the Time 2 measurements also maximized the number of children completing the tests due to learning or practice effects (Rohlman et al., 2005).

Neurobehavioral Statistical Analysis

Neurobehavioral performance measures were summarized using means, standard deviations (SDs), medians, maximum and minimum values. This thesis analysis used regression analysis to assess outcomes related to neurobehavioral test completion and performance. A test completion measure was established as a count of all test measures that were completed. A test was identified as incomplete if a child did not understand instructions or was unmotivated to complete all trials. Test performance was assessed utilizing scores on the overall test battery and individual tests.

Neurobehavioral Test Completion

Multivariable logistic regression was applied to the proportion of neurobehavioral tests completed to assess whether AG children were less likely to complete neurobehavioral tests than Non-AG children after controlling for the child's age, gender and the mother's education. This basic model was expanded to examine potential effects related to pesticide exposure (number of DAPs detected) and the potential interaction between agriculture status and number of DAPs detected. The effect of the interaction was tested (using a likelihood ratio test) and was retained in the model if significant (2-sided $p < 0.10$).

Neurobehavioral Test Performance

As done by Rothlein et al. (2006), a summary index was derived to assess whether the child's performance on the complete battery of tests was associated with agriculture status. To calculate the summary index, measurements for each of the nine neurobehavioral tests were first standardized by subtracting the overall mean for the particular test and dividing the difference by the sample SD. Tests involving latency measures and misses had the signs of the standardized measurements reversed to provide consistency with the other measures. Next, a mean of the standardized measurement was calculated for each test and summed for all nine tests. Multiple linear regression was applied to the summary index to assess whether agriculture status influenced overall neurobehavioral test performance after controlling for the child's age, gender and the mother's education. This basic model was expanded to examine potential effected related to pesticide exposure (number of DAPs detected) and the potential interaction between agriculture status and pesticide exposure. The effect of the interaction was tested using a likelihood ratio test and was retained in the model if significant (2-sided $p < 0.10$). p-values for this comparison are all two-sided.

Multivariable linear regression was also applied to individual test to assess whether individual test performance was associated with agriculture status. However, because the neurobehavioral test battery included multiple tests with multiple measures, a model comparison was conducted to identify tests associated with measures of pesticide exposure. The model comparison consisted of a comparison of adjusted R-squared values between baseline and full model, with a higher R-squared value indicating a better goodness-of-fit. The baseline model included the child's age, gender and mother's education (4 covariates). This baseline model was expanded into a full model that examined potential effects related to agriculture status, number of DAPs detected and potential interactions of the number of DAPs detected with agriculture status, age, and gender (9 covariates). The joint effect of the 2 covariates—agriculture status and the potential interaction of agriculture a status with number of DAPs detected—was tested using an extra sum-of-squares *F*-test. The joint effect of the 4 covariates—number of DAPs detected and the potential interaction of number of DAPs detected with agriculture status, age and gender—was also tested using an extra sum-of-squares *F*-test. Tests based on the *F*-statistic are always two-sided. If one or both of the extra sum-of-squares *F*-test was significant (2-sided $p<0.15$), then the neurobehavioral test was carried forward for additional analysis.

For those neurobehavioral test measures showing potential associations between test performance and agriculture status or pesticide exposure, multivariable linear regression models were developed for each of the neurobehavioral tests. All models retained variables related to the child's age, gender and mother's education, regardless of significance. An extra-sum-of squares *F*-test was again used to test for potential interactions, which were retained in the model if significant (2-sided $p<0.10$).

The potential interaction between the number of DAPs detected with agriculture status was tested to assess whether AG children had more pesticides in their urine, given that higher average concentrations of pesticides were observed in urine of AG children. The potential interactions between the number of DAPs detected with age, and gender were also included to test whether age and/or gender play a role in a child's ability metabolize pesticides. Including these interactions will help to understand the relationship between test performance and the number of DAPs detected may be different for boys and girls and across age groups.

Chapter 3: Results

Demographics

Table 3-1 presents the demographic characteristics of study participants by agriculture status. There were 57 Non-AG children and 124 AG children included in this analysis. The age range of study participants was 4-9 years. The mean age was approximately 6 years and was similar for both Non-AG and AG children. The proportions of males and females were also similar between the two groups. However, the Non-AG group had a higher proportion of males (63%), relative to the agricultural group. The mean number of years of maternal and paternal education was significantly different for Non-AG and AG children. On average, maternal and paternal education was higher for Non-AG children when compared to AG children. Additionally, the minimum and maximum years of education for parents were higher for Non-AG children when compared to AG children.

Table 3-1. Demographics for Non-agricultural (Non-AG) and Agricultural (AG) Children

	Non-AG N=57	AG N = 124	p-value
Age (years)			
Mean (±SD)	5.79 (1.54)	5.70 (1.85)	0.76*
Minimum	4	4	
Maximum	9	9	
N	57	124	
Mother's Education (years)			
Mean (±SD)	9.33 (3.20)	5.80 (3.30)	< 0.01*
Minimum	2	0	
Maximum	14	14	
N	57	123	
Father's Education (years)			
Mean (±SD)	8.85 (3.48)	5.70 (3.41)	<0.01*
Minimum	1	0	
Maximum	14	12	
N	53	105	
Gender			
% Female (N)	37 % (21)	52 % (64)	0.23**
% Male (N)	63 % (36)	48 % (60)	

* two-sided p-value calculated from test of means t-statistic

** two-sided p-value calculated from test of proportions z-statistic

This analysis included children from five Oregon counties: Hood River, Jackson, Lincoln, Multnomah, and Washington. By design, children from Lincoln County did not come from AG households, and were intended to serve as a reference group. Each of the other four counties hosted agricultural industries, and the proportion of children whose parents worked in AG ranged from 41 to 100%. Table 3-2 presents demographics by county. The Lincoln County sample, which only included children from Non-AG households, had the highest levels of maternal and paternal education. In comparison, Washington County, which only included children from AG households, had the lowest levels of maternal and paternal education. Another unique demographic of Lincoln County was that study participants were predominantly male (70%), while other counties were more evenly split with respect to gender.

Table 3-2. Demographics by County

	Hood River	Jackson	Lincoln	Multnomah	Washington
Age (years)					
Mean (±SD)	5.84 (1.95)	4.53 (0.50)	5.2 (0.83)	7.67 (1.06)	5.73 (1.90)
Minimum	4	4	4	5	4
Maximum	9	5	6	9	9
N	37	43	20	30	51
Mother's Education (years)					
Mean (±SD)	6.44 (3.24)	7.58 (3.57)	9.25 (3.18)	8.23 (3.51)	5.02 (3.35)
Minimum	0	0	3	0	0
Maximum	14	14	14	14	12
N	36	43	20	30	51
Father's Education (years)					
Mean (±SD)	6.44 (2.61)	7.05 (4.08)	9.1 (3.21)	8.19 (4.19)	4.6 0(3.01)
Minimum	1	0	3	0	0
Maximum	11	14	14	14	11
N	34	37	20	27	40
Gender					
% Female (N)	57 % (21)	37 % (16)	30 % (6)	50 % (15)	53 % (27)
% Male (N)	43 % (16)	63 % (27)	70 % (14)	50 % (15)	47 % (24)
Agricultural Status					
Non-AG	5 % (2)	44 % (19)	100 % (20)	53 % (16)	0 % (1)
AG	95 % (35)	56 % (24)	0 % (0)	47 % (14)	100 % (51)

Urine Analysis

This study includes urine samples collected at the beginning (Time 1), middle (Time 2) and end (Time 3) of the growing season. The purpose of this analysis was to observe the change in DAPs detected in urine over the growing season and to select a time period that represented the peak exposure of children in subsequent analysis of the relationship to neurobehavioral performance.

Pesticide Exposure Over Time

The two metrics included in this analysis to measure acute levels of pesticide exposure over the growing season were: (1) proportion of DAPs detected and (2) individual concentrations of DAPs detected. Dose of exposure was represented by the proxy "proportion of DAPs detected," with higher proportion representing higher dosage. Duration of exposure was represented by the proxy variable "agriculture status," with AG children representing long-term exposure.

Results for the number of DAPs detected showed differences between Non-AG and AG children (Table 3-3 and Figure 3-1). The difference in the number of detectable concentrations of DAPs in urine was examined across the three time periods for Non-AG and AG children separately. The number of DAPs detected in urine samples was higher at Time 2 than Time 1 for most AG children. The reverse was observed in Non-AG children, in which the number of DAPs was higher at Time 1 than Time 2. Therefore, the distribution of the change in the number of DAPs detected between Time 1 and Time 2 was not significantly different between the two groups (X^2 = 6.328, df=4, p = 0.176). The distribution of the change in the number of DAPs detected between Time 2 and Time 3 was significantly different between the two groups (X^2 = 31.6, df = 4, p < 0.01). The number of DAPs at Time 2 and Time 3 were similar for most AG children; while, most Non-AG children had a fewer DAPs detected at Time 3. Again, the distribution of the number of DAPs detected was significantly different between the two groups between Time 1 and Time 3 ($X^2 = 30.7$, df = 4, $p < 0.01$) (Table 3-3 and Figure 3-1).

The median number of DAPs detected was 1 at all three time points for AG children. For Non-AG children, the median number of DAPs detected was also 1 at Time 1 and Time 2, and 0 at Time 3. The median number of DAPs detected was lowest at Time 2 for both AG and Non-AG children.

Table 3-3 Difference in the number of DAP metabolites detected in urine sample in Non-AG and AG children over Time

	Chi Square Table of # DAPs			
	Decrease	No Change	Increase	P-value
T1 to T2				
Ag	35 (28 %)	37 (30 %)	51 (41 %)	
Non-Ag	24 (41 %)	14 (24 %)	20 (34 %)	0.176
T2 to T3				
Ag	40 (32 %)	44 (36 %)	39 (33%)	
Non-Ag	39 (67 %)	13 (22 %)	6 (10 %)	<0.01
T1 to T3				
Ag	35 (29 %)	37 (31 %)	49 (41 %)	
Non-Ag	43 (46 %)	7 (8 %)	43 (46 %)	<0.01

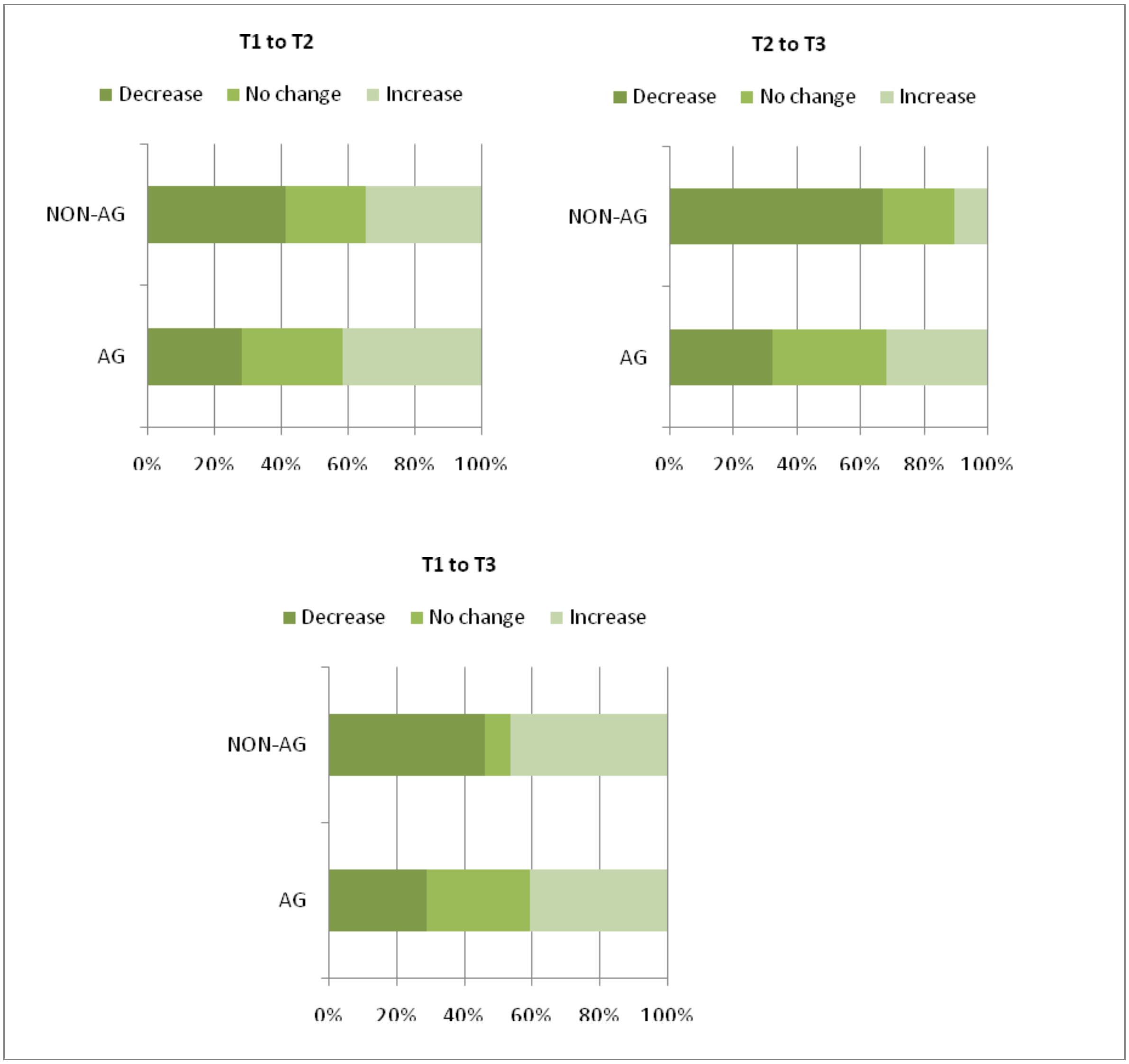

Figure 3-1. Difference in distribution of the change in the number of DAPs Detected Between Time Points for Non-AG and AG children

The second measure used to observe temporal trends was the individual concentration of the five DAPs measured in urine samples. Results presented for this component of the analysis were adjusted for creatinine levels measured in the urine at the time of the sample and reported in microgram per gram of creatinine (μg/g creatinine). Children with creatinine levels <5th percentile and >95th percentile were not reported due to concerns of hydration state and metabolic disorders (Lowewenherz et al., 1997; Lu et al., 2001). Table 3-4 presents the creatinine cut points and the number of samples excluded from this analysis. Note that the sample size varied between the three time points, and Time 3 only included 109 children because urine samples were not collected from children in Jackson County at Time 3.

Table 3-4. Creatinine distribution by Time

Time	5th percentile (N < 5th percentile)	95th percentile (N > 95th percentile)
1	21.5 (8)	155 (8)
2	21 (7)	140 (7)
3	23.5 (6)	160 (5)

Table 3-5 and Figure 3-1 present the geometric means for each DAP at each time point. DMTP was the most frequently detected metabolite at each of the three time points; DEP was the least frequently detected metabolite at all three time points. With the exception of DMTP, the highest geometric mean was observed at Time 2 for both Non-AG and AG children. Concentrations of DMTP appeared to decrease over the growing season, with the highest average concentration observed at Time 1 and the lowest average concentration observed at Time 3. The p-value comparison presented on the right side of Table 3-5 indicates whether or not there was a statistically significant difference between the two time points. General changes in creatinine-adjusted DAP concentrations across the growing season were not statistically significant ($p>0.05$).

With the exception of DETP, the geometric means for the DAP metabolites were higher for AG children than Non-AG children. The largest difference in average concentrations between the two groups was observed at Time 2 for DMP concentrations higher than non-agriculture children by 2.97 μg/g creatinine. The average DETP concentration was higher for Non-AG children at Time 2 than AG children by a small amount (0.33 μg/g creatinine).

Table 3-5. Creatinine-corrected DAP metabolite levels (μg/g creatinine) in urine samples of Non-AG and AG children over time

	Geometric Mean (95% CI)			*p*-value† comparison of DAP levels		
	T1 N=114	T2 N=150	T3 N=109	T1-T2	T2-T3	T1-T3
DMP						
Non-AG	4.65 (3.54, 6.10)	4.00 (3.15, 5.07)	5.25 (3.67, 7.50)	0.130	0.152	0.230
AG	5.13 (4.19, 6.29)	6.97 (5.55, 8.76)	5.41 (4.19, 7.00)	0.273	0.280	0.134
Non-AG + AG	4.98 (4.23, 5.86)	5.92 (4.95, 7.08)	5.37 (4.36, 6.61)	0.679	0.586	0.066
DMTP						
Non-AG	6.24 (3.97, 9.80)	4.86 (2.80, 8.43)	4.77 (2.58, 8.83)	0.359	0.788	0.283
AG	8.87 (6.36, 12.38)	7.08 (5.27, 9.50)	6.80 (4.70-9.83)	0.193	0.941	0.540
Non-AG + AG	7.97 (6.10, 10.41)	6.34 (4.88, 8.23)	6.19 (4.53-8.46)	0.109 (-1.16)	0.939	0.288
DMDTP						
Non-AG	1.65 (1.17, 2.32)	1.74 (1.25, 2.42)	2.25 (1.45, 3.49)	0.636	0.081 (-1.22)	0.285
AG	2.07 (1.62, 2.64)	3.45 (2.63, 4.53)	2.16 (1.59, 2.93)	0.062 (1.18)	0.04 (1.24)	0.979
Non-AG + AG	1.93 (1.58, 2.35)	2.83 (2.27, 3.52)	2.18 (1.70, 2.80)	0.064 (1.15)	0.163	0.617
DEP						
Non-AG	1.22 (1.06, 1.40)	1.57 (1.30, 1.90)	2.00 (1.45, 2.76)	0.003 (1.11)	0.279	0.09
AG	1.77 (1.53, 2.04)	2.09 (1.78, 2.44)	2.03 (1.63, 2.53)	0.35	0.362	0.853
Non-AG + AG	1.58 (1.41, 1.76)	1.92 (1.69, 2.17)	2.02 (1.69, 2.42)	0.081 (1.06)	0.694	0.578
DETP						
Non-AG	1.23 (0.96, 1.58)	2.31 (1.47, 3.62)	1.71 (1.19, 2.48)	0.019 (1.36)	0.793	0.882
AG	1.97 (1.58, 2.47)	1.98 (1.56, 2.53)	1.80 (1.40, 2.30)	0.439	0.718	0.625
Non-AG + AG	1.71 (1.44, 2.03)	2.07 (1.67, 2.57)	1.77 (1.45, 2.18)	0.500	0.653	0.717

[1] Age 6-11, no data because geometric mean not calculated when the proportion of detects was high (CDC, 2005).

*Excluded children with creatinine levels < 5th and > 95th percentile

† If p-value is significant ($P<0.10$), then the beta coefficient is listed in parenthesis

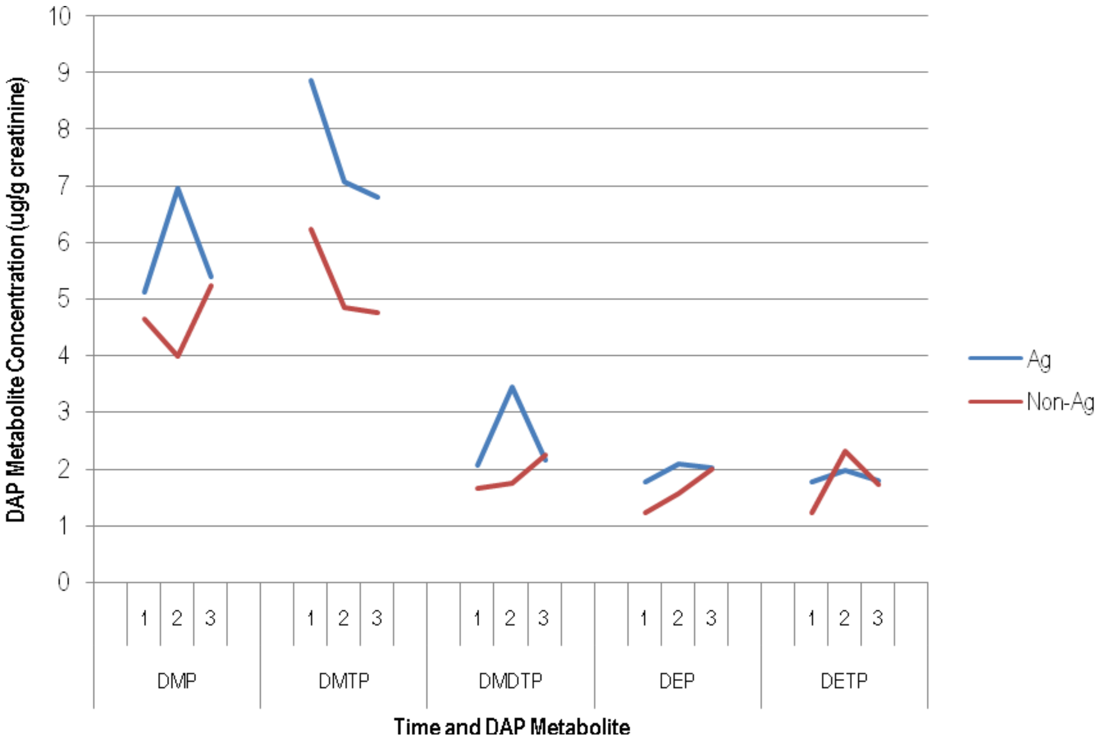

Figure 3-2. Geometric mean DAP metabolite concentration over time

Relationship Between Agriculture Status and Pesticide Exposure at Time 2

Based on urine sample results, Time 2 was selected for subsequent analysis. The rationale for selecting Time 2 included the following:

- The estimated proportion of DAPs detected was significantly different between the two groups at Time 2
- The highest geometric means were observed at Time 2 for most DAPs; and
- The largest sample size was obtained at Time 2

Table 3-6 presents a statistical summary of DAP concentrations in urine samples measured at Time 2. The percent of samples below limits of detection (non-detect") was higher for non-agriculture children than agriculture children for all DAP metabolites, except

DETP. National geometric means were available for comparisons involving DMTP, DEP, and DETP. The Oregon sample of children as a whole revealed higher geometric mean concentrations of DAP metabolites when compared to the national average (CDC, 2005). The Non-AG geometric mean was slightly higher and the AG geometric mean was significantly higher than the NHANES estimates for DMTP and DETP. It should be recognized that the elevated concentrations observed in our analysis of urine samples from Oregon children might be an artifact of the high proportion of non-detects in our sample and the high LODs. A value of one-half the LOD was substituted for non-detects was used in geometric mean calculations.

Table 3-6. DAP metabolite levels (μg/g creatinine) in urine samples of Non-AG and AG children at Time 2

	Non-Detected (%)	Median	Min	Max	Geometric Mean [95% CI]	NHANES[1] Geometric mean [95% CI]
DMP						
Non-AG	80 %	3.28	1.67	72.86	4.00 [3.15, 5.07]	
AG	59 %	4.66	1.43	97.89	6.97 [5.55, 8.76]	
Non-AG + AG	65 %	4.00	1.43	97.89	5.92 [4.95, 7.08]	No data
DMTP						
Non-AG	67 %	2.06	0.92	535.21	4.86 [2.80, 8.43]	
AG	45 %	6.61	0.79	452.3	7.08 [5.27, 9.50]	
Non-AG + AG	51%	4.15	0.79	535.21	6.34 [4.88, 8.23]	2.95 [2.25, 3.86]
DMDTP						
Non-AG	84 %	1.14	0.57	63.6	1.74 [1.25, 2.42]	
AG	61 %	1.98	0.57	100	3.45 [2.63, 4.53]	
Non-AG + AG	68 %	1.60	0.57	100	2.83 [2.27, 3.52]	No data
DEP						
Non-AG	95 %	1.35	0.71	13.1	1.57 [1.30, 1.90]	
AG	89 %	1.75	0.71	54.55	2.09 [1.78, 2.44]	
Non-AG + AG	91 %	1.51	0.71	13.1	1.92 [1.69, 2.17]	1.43 [0.87, 2.34]
DETP						
Non-AG	79 %	1.25	0.57	243	2.31 [1.47, 3.62]	
AG	84 %	1.37	0.57	909	1.98 [1.56, 2.53]	
Non-AG + AG	83 %	1.36	0.57	909	2.07 [1.67, 2.57]	0.59 [0.47, 0.74]

[1] Age 6-11, no data because geometric mean not calculated when the proportion of non-detects was > 60% (CDC, 2005).
*Exclude children with creatinine levels < 5th and > 95th percentile

Multivariable linear regression analysis of concentrations of individual DAP metabolites did not demonstrate an association with agriculture status and the urine sample concentrations of DMP, DMDTP, DEP, or DETP, after adjusting for age, gender and maternal education (Appendix A).

Multivariable logistic regression analysis of the proportion of DAPs detected in urine samples presented significant findings (Table 3-7). This model demonstrated that AG children were twice as likely to have a greater number of DAPs detected in their urine. No interactions were significant in the model (Likelihood Ratio Test X^2 =3.09, df=3, *p* = 0.38). County was a significant variable in the model (Likelihood Ratio Test X^2 =9.90, df=4, *p* = 0.04). The model results for county were not expected, revealing that children residing in the agricultural counties were less likely to have more DAPs detected in their urine relative to the Non-AG children in Lincoln County.

Table 3-7. Relationship between the proportion of DAP metabolites detected and agricultural status adjusted for gender, age, maternal education and county

	β (SE)	OR [95% CI]	p-value
Agricultural Status			
Non-Ag	Reference		
Ag	2.40 (0.56)	1.97 [1.13, 3.44]	0.02
Gender			
Male	Reference		
Female	-2.02 (0.12)	0.70 [0.50,0.99]	0.04
Age group			
<6 years	Reference		
≥6yrs	-0.46 (0.20)	0.90 [0.58, 1.39]	0.65
Maternal education			
<9 yrs	Reference		
9-12 yrs	0.47 (0.25)	1.11 [0.71, 1.74]	0.64
>12 yrs	-2.08 (0.15)	0.57 [0.33, 0.97]	0.04
County			
Hood River	Reference		
Jackson	1.89 (0.47)	1.69 [0.98, 2.91]	0.06
Lincoln	1.59 (0.82)	1.95 [0.86, 4.46]	0.11
Multnomah	-1.37 (0.22)	0.61 [0.30, 1.24]	0.17
Washington	-0.33 (0.22)	0.92 [0.58. 1.48]	0.74

Table 3-8 shows that the estimated proportion of pesticide metabolites by county tended to be higher for AG children. Our sample, by design, does not include AG children in Lincoln County, and only one Non-AG child in Washington County; therefore, odds ratios could not be calculated for these two counties. AG children in Jackson County are more than twice as likely to have more DAPs detected in urine, than Non-AG children in Jackson County (*p*<0.01).

Table 3-8. Estimated Proportion (95%CI) of DAP metabolites detected in urine of Non-AG and AG children by county at Time 2

	Est. Proportion (n) [95% CI]		OR	p-value
	Non-Ag	Ag		
Hood River	0.20 (1) [0.28, 0.68]	0.28 (31) [0.20, 0.33]	1.54	0.71
Lincoln	0.28 (16) [0.18, 0.36]	-- (0) [--]	--	--
Jackson	0.24 (18) [0.16, 0.33]	0.42 (21) [0.32, 0.50]	2.23	0.01
Multnomah	0.08 (8) [0.03, 0.19]	0.20 (12) [0.12, 0.32]	3.08	0.10
Washington	0.00 (1) [--]	0.27 (42) [0.21, 0.32]	--	--

Note:
-- = inadequate sample size to calculate value

Table 3-9 presents the multivariable regression results without county and demonstrate that AG children did not have significantly more DAPs detected in their urine (p = 0.13).

Table 3-9. Relationship between the number of DAP metabolites detected and agricultural status adjusted for gender, age, maternal education

	β (SE)	OR [95% CI]	p-value
Agricultural Status			
Non-Ag	Reference		
Ag	1.50 (0.28)	1.36 [0.91, 2.04]	0.13
Gender			
Male	Reference		
Female	-2.61 (0.11)	0.64 [0.46, 0.90]	0.01
Age group			
<6 years	Reference		
≥6yrs	-2.47 (0.11)	0.64 [0.45, 0.91]	0.01
Maternal education			
<9 yrs	Reference		
9-12 yrs	0.90 (0.26)	1.22 [0.79, 1.86	0.37
>12 yrs	-2.19 (0.15)	0.56 [0.33, 0.94]	0.03

Neurobehavioral Test Statistical Analysis

Summary statistics for Time 2 of the neurobehavioral data are presented in Table 3-10. On average, Non-AG children performed better than AG children on Match-to-Sample, Finger Tapping, Divided Attention Words/Language, Object Memory, Purdue Pegboard and Visual Motor Integration tests. On average, Non-AG and AG children performed similarly on Digit Span and Object Memory. AG children performed better than Non-AG children on Continuous Performance Hits Measures.

Table 3-10. Summary Statistics for Neurobehavioral Test Results of Non-AG and Ag children at Time 2

NB Test *(possible score range)*	Non-AG				AG			
	Mean ± SD	Median	Min	Max	Mean ± SD	Median	Min	Max
Digit Span *(0-9)*	4 (0.89)	4	3	6	4 (0.92)	4	3	6
Match-to-Sample								
Number correct *(0-45)*	10 (3)	11	4	15	10 (3.6)	10	1	15
Latency* *(>100 – 10,000 msec)*	4905 (1543)	4736	2164	9318	5311 (2002)	4956	2659	12083
Finger Tapping								
Number of taps right	57 (15)	57.5	34	98	56 (15)	53	28	100
Number of taps left	49 (11)	49	29	77	48 (13)	45	24	88
Continuous Performance								
Hits	24 (6)	25.5	3	30	25(5)	26	1	30
Misses*	6 (6)	4.5	0	26	5 (5)	3	0	19
Percent of false alarms*	5 (3)	5	0	14	4 (4)	2	0	17
Latency*	557 (133)	561	291	887	585 (153)	599	248	994
Percent of corrected hits	79 (20)	85	10	98	83 (16)	90	34	98
Percent of corrected false alarms*	12 (7)	11	1	31	8 (9)	4	1	38
d prime	2.2 (0.92)	2.27	0.58	4.4	2.7 (1)	2.8	0.11	4.4
Divided Attention Words/Language								
Right hand taps	48 (13)	48	17	75	46 (12)	44	21	76
Left hand taps	43 (11)	43	24	63	39 (10)	37.5	15	67
Right hand song	34 (14)	30	13	68	31 (12)	29	12	72
Left hand song	33 (12)	33	9	57	32 (11)	30	9	63
Right hand *(0-60)*	0.78 (0.20)	0.816	0.11	1.2	0.79 (0.20)	0.759	0.33	1.26
Left hand *(0-60)*	0.82 (0.23)	0.85	0.19	1.26	0.82 (0.21)	0.812	0.30	1.48
Control *(0-60)*	0.95 (0.29)	1	0.34	1	0.96 (0.20)	0.93	0.47	1.63
Symbol-digit								
Latency* *(>100 - ~15,000 msec)*	4857 (1553)	4229	3198	8979	4411 (1429)	4323.5	2137	7873
Errors* *(0-45)*	3.6 (1.9)	3	1	7	2 (2)	2	0	8
Object Memory								
Number of items named *(0-18)*	15 (1.7)	15	7	16	15 (1.5)	15	9	16
Immediate recall *(0-18)*	7 (2)	7	3	11	8 (2)	8	2	12
Delayed recall *(0-18)*	6.2 (2.4)	7	1	11	6 (2.8)	6	0	11
Recognition *(0-18)*	14 (3.8)	16	3	16	14 (4)	16	-1	16
Purdue Pegboard								
Number of pegs right *(0-25)*	11 (3)	11.5	5	17	11 (3)	10.5	6	18
Number of pegs left *(0-25)*	10 (2)	7.5	7	17	10 (3)	7	5	17
Number of pegs both *(0-25)*	7 (2)	9.5	5	13	8 (3)	9.25	15	13
Visual Motor Integration *(0-18)*	11 (3.5)	12	5	17	11 (3)	10	2	18

Notes: * Lower score indicates better performance

Neurobehavioral Test Completion Results

A child's ability to complete a neurobehavioral test can be a measure of the child's attention in which the child understands the text but was not motivated to complete the test. A child's ability to complete a neurobehavioral test can also be a measure of development, in which the child may not be able to understand the test concept well enough to complete the test. Agriculture status and the number of pesticide metabolites were not associated with a child's ability to complete the tests in this study. However, as expected, age was a significant predictor of a child's ability to complete the test battery. Children over the age of 6 years were more likely to complete the tests than children under the age of 6 yrs (OR = 7.6 [95% CI: 4.9, 11.8]). Table 3-11 presents the results of this comparison. The interaction between agriculture status and the number of DAPs detected was not significant in the model (Likelihood Ratio Test $X^2 = 0.79$, df = 1, $p = 0.37$).

Table 3-11. Relationship between the proportion of neurobehavioral tests completed out of nine tests and agriculture status, adjusted for gender, age, and maternal education

	β (SE)	OR [95% CI]	p-value
Agricultural Status			
Non-Ag	Reference		
Ag	0.07 (0.17)	1.07 [0.77, 1.49]	0.70
Gender			
Male	Reference		
Female	-0.01 (0.15)	0.99 [0.74, 1.32]	0.93
Age group			
<6 years	Reference		
≥6yrs	2.03 (0.22)	7.61 [4.91, 11.81]	0.00
Maternal education			
<9 yrs	Reference		
9-12 yrs	-0.06 (0.19)	0.94 [0.64, 1.38]	0.76
>12 yrs	-0.11 (0.20)	0.89 [0.61, 1.32]	0.57

Overall Neurobehavioral Test Performance Results

Results of the multivariable linear regression for the summary index of neurobehavioral tests demonstrated agriculture status and pesticide exposure were not significantly associated with overall performance. Children over the age of 6 years were more likely to perform better on neurobehavioral tests than children less than 6 years of age, consistent with the pattern observed for test completion. Results also revealed males performed better than females on the overall test battery. Interactions of agriculture status and number of DAPs, and age and gender were not significant ($F_{3, 151} = 1.47$, $p = 0.22$). Results are presented in Table 3-12.

Table 3-12. Relationship between the overall neurobehavioral tests performance and agriculture status, adjusted for gender, age, and maternal education

	β (SE)	[95% CI]	p-value
Agricultural Status			
Non-Ag	Reference		
Ag	-0.33 (0.56)	[-1.44, 0.78]	0.56
Gender			
Male	Reference		
Female	-1.25 (0.47)	[-2.19, -0.32]	0.01
Age group			
<6 years	Reference		
≥6yrs	7.22 (0.48)	[6.27, 8.18]	0.00
Maternal education			
<9 yrs	Reference		
9-12 yrs	0.34 (0.62)	[-0.88, 1.55]	0.59
>12 yrs	0.46 (0.71)	[-0.94, 1.86]	0.52

Individual Neurobehavioral Test Performance Results

Table 3-13 shows a comparison of the two linear regression models fit for each neurobehavioral test. In the baseline model the neurobehavioral test was the response variable and age, gender, and maternal education were the explanatory variables. In the full model, the response variable was the same and the explanatory variables were expanded to include agriculture status, number of DAP metabolites detected in urine and three interactions. The interactions included in the full model were the interaction of agriculture status and DAP metabolites detected, and the interactions of the number of DAPs with age and gender. Table 3-13 presents the adjusted R-squared for the baseline model for each of the individual test measures. The extra sum of squares F-test indicated whether or not agriculture status and the number of DAP metabolites was significant ($p<0.15$).

Table 3-13. Neurobehavioral test performance model comparison

	N	Baseline Model Adj. R^2 (%) [4 df]	Full Model Adj. R^2 (%) [9 df]	Is Agricultural Status significant?* (2 df F-test p-value)	Is # of DAPs detected significant?** (4 df F-test p-value)
Digit Span	133	37.71 %	37.04%	No (0.30)	No (0.46)
Match-to-Sample					
Number correct	145	31.15%	30.54%	No (0.53)	No (0.45)
Latency	145	19.14%	19.16%	No (0.42)	No (0.55)
Finger Tapping					
Number of taps right	159	45.03%	45.39%	No (0.78)	No (0.21)
Number of taps left	160	44.14%	48.09%	No (0.85)	Yes (0.00)
Continuous Performance Hits					
Hits	122	-0.56%	2.06%	Yes (0.02)	Yes (0.15)
Misses	122	-0.28%	2.56%	Yes (0.02)	Yes (0.15)
Percent of false alarms	122	11.10%	9.82%	No (0.27)	No (0.94)
Latency	122	31.15%	29.82%	No (0.79)	No (0.67)
Percent of corrected hits	122	-0.43%	2.45%	Yes (0.02)	Yes (0.14)
Percent of corrected false alarms	122	10.80 %	9.56 %	No (0.27)	No (0.94)
Continuous Performance Hits d prime	122	3.37 %	5.00 %	Yes (0.05)	No (0.70)
Divided Attention Words/Language					
Right hand taps	142	33.10 %	36.38 %	No (0.78)	Yes (0.020)
Left hand taps	143	33.62 %	39.67 %	Yes (0.09)	Yes (0.00)
Right hand song	142	13.56 %	12.68 %	No (0.64)	No (0.52)
Left hand song	143	29.05 %	29.21 %	No (0.57)	No (0.28)
Right hand	142	-0.64 %	-1.33 %	No (0.77)	No (0.41)
Left hand	143	0.22 %	-0.26 %	No (0.59)	No (0.38)
Control	142	1.92 %	-0.17 %	No (0.44)	No (0.76)
Symbol-digit					
Latency	51	-2.53 %	1.95 %	No (0.51)	No (0.28)
Errors	51	-1.00 %	3.93 %	No (0.15)	No (0.45)
Object Memory					
Number of items named	158	15.11 %	14.87 %	No (0.18)	No (0.34)
Immediate recall	158	18.58 %	18.45 %	Yes (0.11)	No (0.99)
Delayed recall	158	5.99 %	2.91 %	No (0.98)	No (0.99)
Recognition	143	6.15 %	8.41 %	No (0.71)	Yes (0.10)
Purdue Pegboard					
Number of pegs right	157	57.03 %	58.41 %	Yes (0.10)	Yes (0.06)
Number of pegs left	154	50.84 %	52.66 %	No (0.39)	Yes (0.04)
Number of pegs both	151	50.85 %	52.45 %	No (0.23)	Yes (0.08)
Visual Motor Integration	162	44.85 %	46.38 %	No (0.23)	Yes (0.13)

Notes:
Baseline Model includes age group [df = 1], maternal education group [df = 2], gender [df = 1]
Full Model includes age group [df = 1], maternal education group [df = 2], gender [df = 1], agriculture status [df = 1], number of DAPs detected [df = 1], and 3 interactions (agriculture status x number of DAPs detected [df = 1]; age group x number of DAPs detected [df = 1]; and gender x number of DAPs detected [df = 1])
Shading indicates agriculture and/or # of DAPs detected is statistically significant ($P < 0.15$)
* Simultaneous F-test agriculture status and agriculture status x number of DAPs detected
** Simultaneous F-Test number of DAPs detected, agriculture status x number of DAPs detected, age group x number of DAPs detected, and gender x number of DAPs detected

Table 3-13 was used to screen NB test results for association with either agriculture status and/or DAPs detected in urine. Results identified six neurobehavioral tests potentially associated with the two covariates of interest and these neurobehavioral tests were assessed individually. Tables 3-14 present multivariable linear regression model results for each of these six tests.

Table 3-14. Neurobehavioral test associated with pesticide exposure

			Baseline Model [4 df]			Full Model				
	N	Adj. R^2 (%)	Age Group [1 df]	Mother Ed Group [2 df]	Sex [1 df]	AG Status [1 df]	# DAPs [1 df]	AG Status x # DAPs [1 df]	# DAPs x Age Group [1 df]	# DAPs x Sex [1 df]
Finger Tapping										
Number of taps left	160	49 %	•	•	•		•		•	•
Continuous Performance Hits										
Hits	122	4 %	•	•	•	•	•	•		
Misses	122	4 %	•	•	•	•	•	•		
Percent of corrected hits	122	4 %	•	•	•	•	•	•		
Divided Attention Words/Language										
Right hand taps	142	38 %	•	•	•		•			
Left hand taps	143	40 %	•	•	•	•	•			•
Object Memory										
Immediate recall	158	20 %	•	•	•	•				
Recognition	143	10 %	•	•	•		•			•
Purdue Pegboard										
Number of pegs right	157	58 %	•	•	•	•	•	•		
Number of pegs left	154	52 %	•	•	•		•			
Number of pegs both	151	52 %	•	•	•		•			
Visual Motor Integration	162	46%	•	•	•		•			

• = Variable included in the model
Shaded cells indicates variable significant in the model (*P*<0.05*)*
Blank cells variable not included in the model (1 df F-test, *P*<0.10)

As expected, neurobehavioral test performance was better in children ≥ 6 years of age, when compared to children < 6 years of age. Age was also shown to be associated with DMTP concentrations (see Table A-3) As a result, age is a significant predictor in the relationship between neurobehavioral test performance and pesticide exposure. Other variables included in the Baseline Model, maternal education and gender, were not consistently found to be associated with neurobehavioral test performance. Gender was associated with a child's performance on the Divided Attention Right Hand Tap measure and males were more likely to perform better than females.

An interaction between gender and the number of DAPs detected was observed for the Left Hand Finger Tapping test. Figure 3-3 presents this interaction for males <6 years, females < 6 years, males ≥ 6 years and females ≥ 6 years. Older males and females performed significantly better than younger males and females ($p<0.01$). As the number of DAPs detected in urine increased, females tended to perform worse and males tend to perform better.

An interaction between agriculture status and the number of DAPs detected was found in three of the continuous performance measures (Figures 3-4 to 3-6). Among children with more than one DAP detected in their urine, AG children performed worse than Non-AG children. For children with less than 1 DAP detected in their urine sample, AG children performed better than Non-AG children. This interaction was observed for all levels of maternal education, across <6 and ≥ 6 year age groups, and for both genders.

For the Divided Attention Left Hand Finger Tap Non-AG children performed better than AG children (Figure 3-7). Boys tended to perform better than girls; as the number of DAPs detected increased, girls tended to perform the same, while boys tended to perform better ($p<0.01$). Similar trends were observed in the Object Memory Recognition test (Figure 3-8).

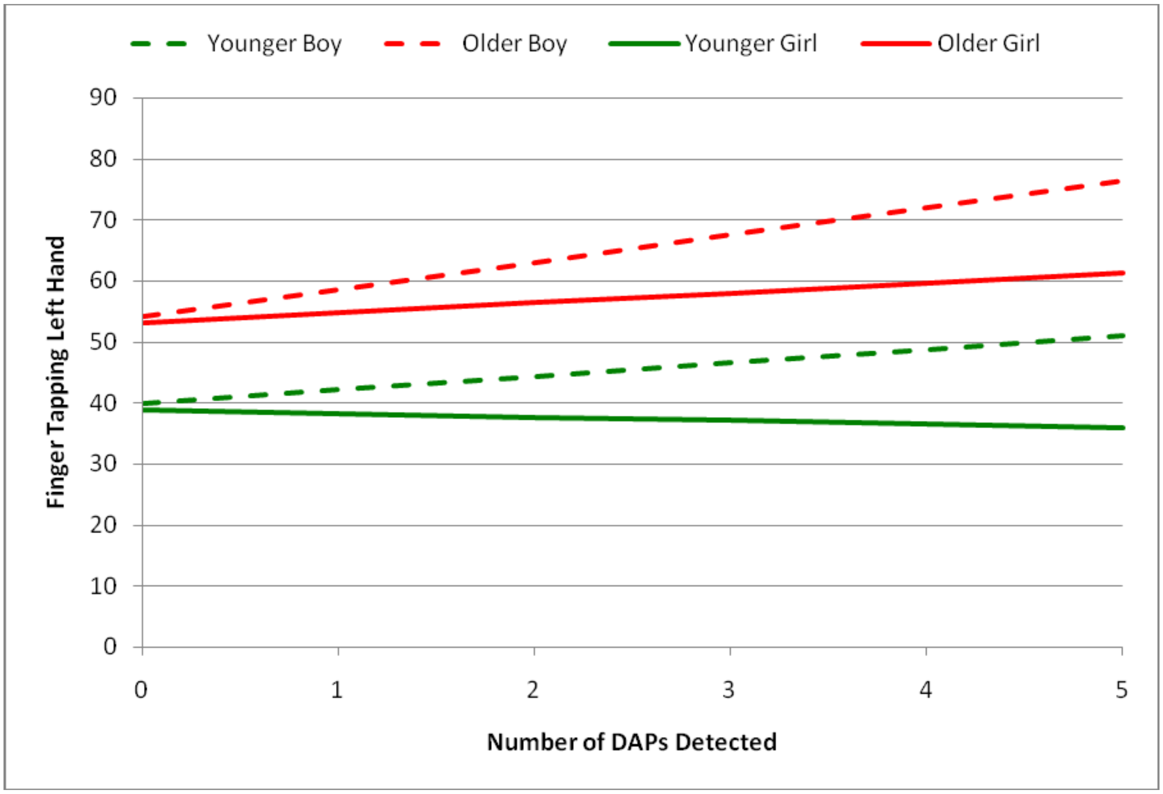

Figure 3-3. Finger Tapping Number of Taps Left: Schematic depiction of interaction between gender and number of DAPs detected in urine samples for boys and girls

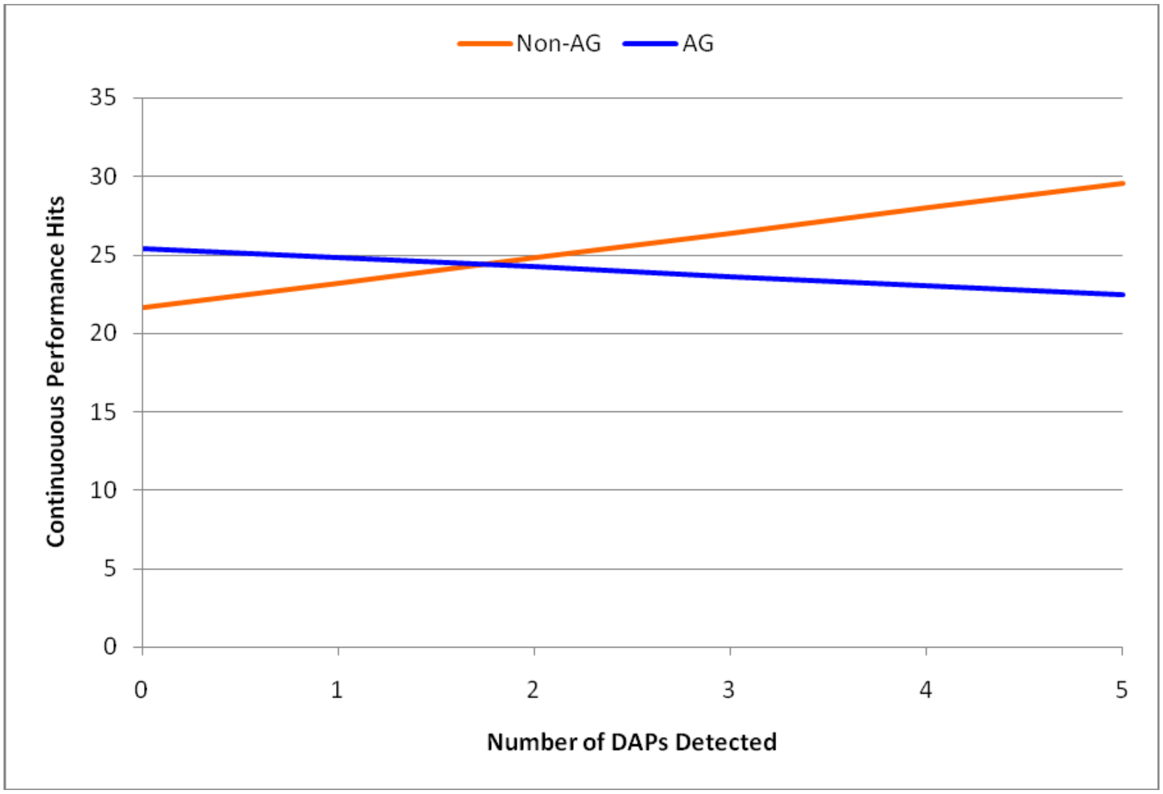

Figure 3-4. Continuous Performance Hits: schematic depiction of interaction between agriculture status and the # of DAPs detected

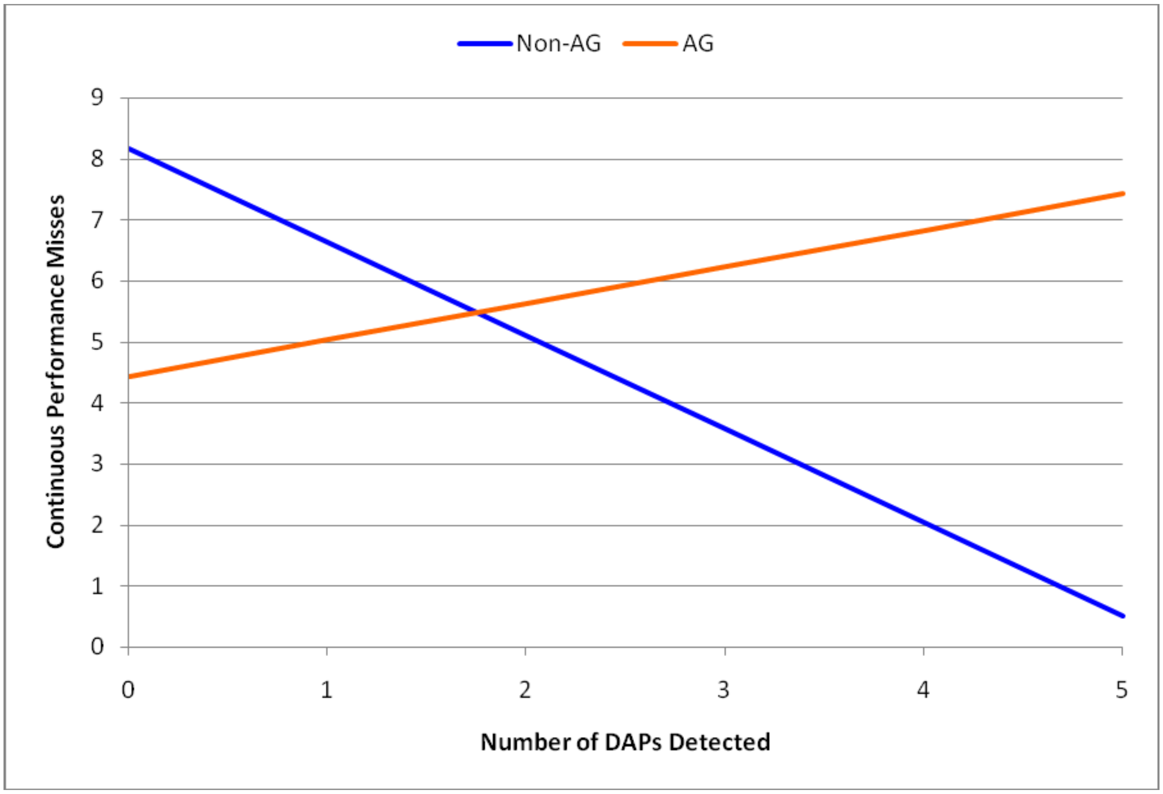

Figure 3-5. Continuous Performance Misses: Schematic depiction of interaction between agriculture status and the # of DAPs detected

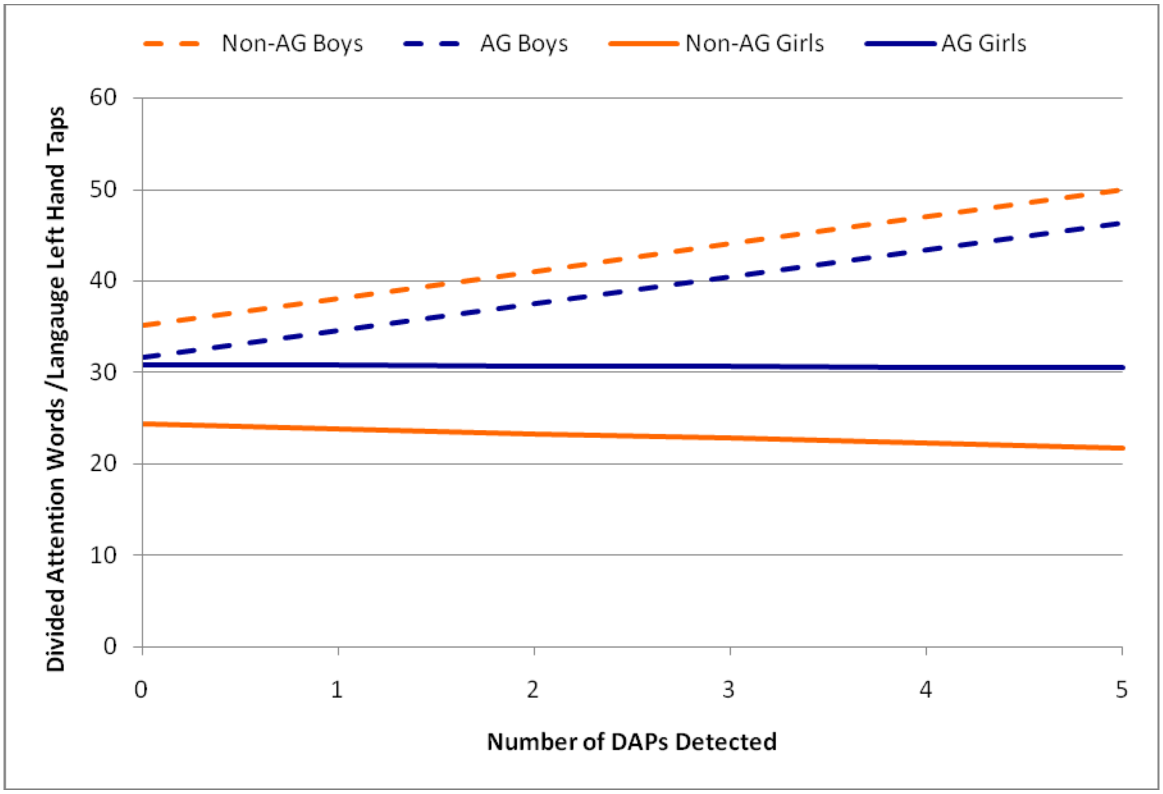

Figure 3-6. Divided Attention Words/Language Left Hand Taps: Schematic depiction of interaction between gender and number of DAPs detected in urine samples for boys and girls

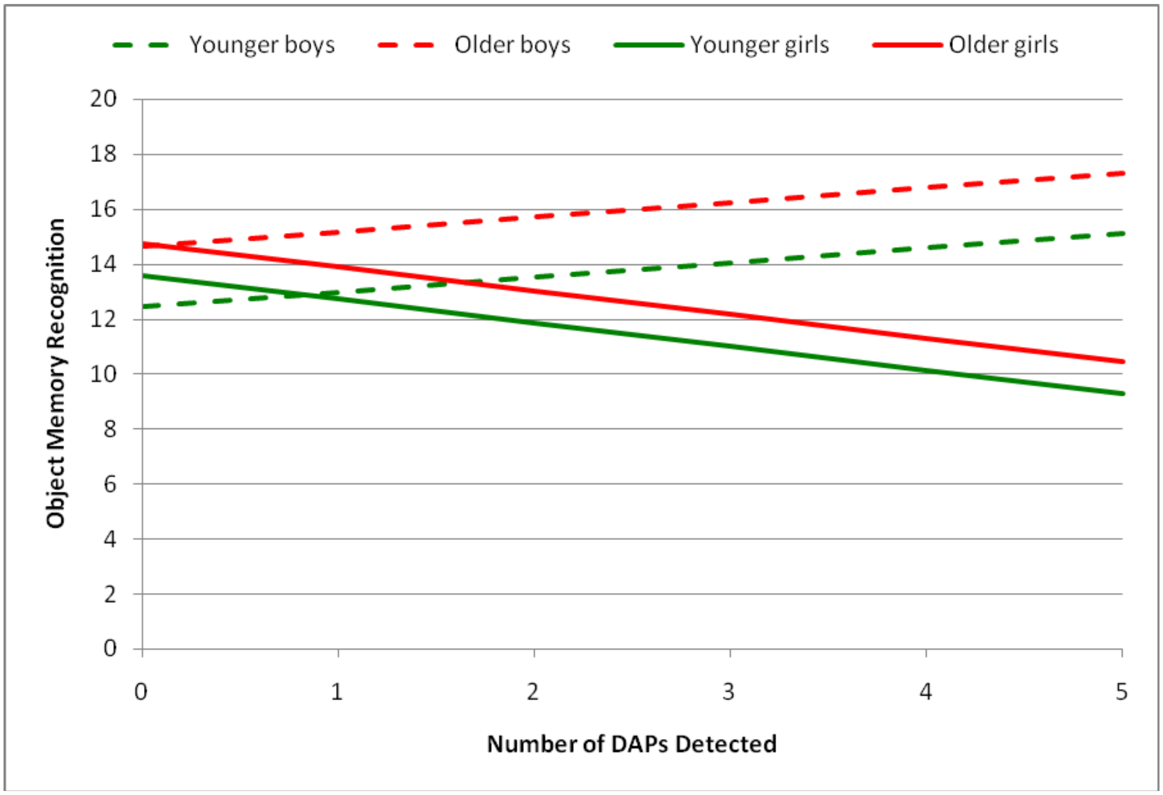

Figure 3-7. Object Memory Recognition: Schematic depiction of interaction between gender and number of DAPs detected in urine samples for boys and girls

Chapter 4: Discussion

This analysis has demonstrated that children of agriculture workers are more likely to be exposed to OP pesticides than children of non-agriculture workers. Within the AG group, the highest concentrations of individual DAP metabolites were detected at the middle of the growing season when exposure to pesticide residues was expected to be high. AG children with a greater number of DAP metabolites in their urine samples performed worse on some neurobehavioral tests. These observations were made across several counties in Oregon hosting differing agricultural industries and types of crops.

Pesticide Exposure

Urinary biomarkers were used in this analysis to represent levels of recent exposure, while parents history of work in agriculture was used to classify long-term exposure. DAP metabolites were present in urine of both AG and Non-AG children in very low concentrations. When concentrations were at detectable levels, AG children had higher concentrations and more detectable DAP metabolites. Given these two observations, children of agriculture workers likely experienced more exposure to OPs than children of non-agriculture workers. These findings were expected and are consistent with the findings of another Northwest investigation of OP pesticides in farm worker families (Lu et al., 2000).

This difference in detectable DAP metabolites between the two groups may be a reflection of the additional pesticide exposure experienced by agricultural communities. For some DAP metabolites the highest mean concentrations were observed at the middle of the growing season. This pattern observed in the urinary biomarkers appears to match the patterns of use in the community. At the beginning of the growing season, pesticide residues begin to be transferred from the parent's work environment to the home environment. DAP metabolites were detected at higher concentrations in the AG children than Non-AG children at the beginning of the season. Because the population under study is migratory, parents may have already been working in agriculture areas in California and children may have received carry home exposures prior to coming to Oregon. As the growing season progresses, pesticide residues may accumulate in the home. As a result, higher concentrations were observed in the middle of the season. Toward the end of the season, parent's workload may taper off, and may result in fewer pesticide residues transferred from the fields to the home environment. Toward the end of the season, as harvest time nears, OP pesticide application may no longer be needed to control for insect damage in crops, as a results workers are exposed to fewer pesticides and fewer pesticide residues are transferred from the field to the home environment. Additionally, OP pesticide residues degrade over time due to moisture, sunlight, and microbial activity.

One unexpected finding in this analysis, was the effect of county on the relationship between pesticide exposure and agriculture status. The effect of county on the number of DAP metabolites detected in urine was contrary to the hypothesis that children residing in agricultural counties would be exposed to more pesticides via proximity of residences and carry-home processes. Results suggest that children living in Lincoln County were exposed to more pesticides than children in Jackson County. The data also revealed that there was no difference in the average number of pesticide metabolites detected in Lincoln County non-agriculture children and Hood River agriculture children. There were no agriculture children in Lincoln County for comparison, but all other counties demonstrated greater risk of pesticide exposure in agriculture children than non-agriculture children. Both Jackson County and Multnomah County included children from both agriculture and non-agriculture groups, and for Jackson County the sample size was large enough to achieve statistical significance. Within Jackson County alone, agriculture children were twice as likely to have a greater number of DAP metabolites detected in their urine compared to non-AG children.

DAP metabolites detected in urine represent a measure of daily exposure to OPs. Barr et al. (2004) pointed out that caution should be used when using creatinine-adjusted concentrations to account for variable dilution, because of age and ethnicity differences. This concern may be less of an issue in this analysis given that all of the study participants were Hispanic children between the ages of 4 and 11 years.

Results also suggest that the average DMTP, DEP and DETP concentrations measured in boys and girls of Oregon farm worker families are higher than the national average reported for children age 6-11 in the 2001-2002 NHANES (CDC, 2005). The 2001-2002 NHANES participants are representative sample of the general US population ages 6-11 years. The NHANES is the best available estimate of US values for children. Although the NHANES sample includes children with AG exposure, the proportion of children whose parents work in agriculture would be expected to be small.

Neurobehavioral Tests and Pesticide Exposure

On average Non-AG children performed better on neurobehavioral tests than AG children. However, AG children with a greater number of DAPs in their urine samples had more difficulty performing tests involving attention (Continuous Performance) and dexterity. Similar findings have been published in the literature. A study of children in the US exposed to methyl parathion, a restricted OP pesticide used for pest control, demonstrated similar findings (Ruckart et al., 2004). Exposure was not associated with deficits in most neurobehavioral tests; however, exposed children had more difficulty with tests involving attention.

A subset of the children included in this study were evaluated by Rohlman and colleagues (2005) who identified specific deficits on measures of motor speed and coordination in AG children when compared to Non-AG children. Continuous performance test results were not reported given the observed difficulties in test completion. A child's ability to complete a test is itself a measure of attention and the observation that children experienced difficulties in this test may be an indication of attention deficits. Therefore, in the present analysis we considered completion of continuous performance tests as an outcome. This analysis used a larger sample size and demonstrated deficits in attention measurements for AG children exposed to more than one DAP metabolite. Interestingly, findings in children age 4-11 are similar to early studies of adult populations with long term, low-level exposure to OP pesticides. These studies reported deficits in measures of sustained attention, information processing and motor speed and coordination (Stephens et al. 1995; Fiedler et al. 1997; Bazylewicz-Walczak et al., 1999).

Interactions observed in multivariate regression models suggested that AG children did not perform as well as Non-AG children when more DAP metabolites were detected in urine. When fewer DAP metabolites were detected in urine, Non-AG children performed similarly and sometimes AG children performed better than Non-AG children. Better test performance by AG children may be the result of AG children being recruited from the Migrant Head Start program. Given the educational advantage of being enrolled in Migrant Head Start, these AG children may perform better on the neurobehavioral tests regardless of pesticide exposure.

Potential Limitations

The likelihood that bias, residual confounding and/or chance play a role in the relationships observed in this analysis are discussed below.

Bias

Selection bias may influence the outcome of this analysis. The agriculture children were recruited from a migrant head start program, and the non-agriculture children were recruited from the community. This differential selection bias would be expected to obscure deficits in neurobehavioral test results caused by OP exposure.

This analysis may also be subject to misclassification bias, as the classification of pesticide exposure by DAP metabolites in urine has limitations. DAP metabolites have a short half-life of 24-48 hrs and reflect recent exposure. By combining the DAP metabolites into one measure of the number of DAP metabolites detected, as opposed to individual concentrations, this study attempts to avoid the potential for exposure misclassification. In addition, a long-term exposure metric is considered by classifying children as AG and Non-AG.

DAP metabolites in urine are not specific measurements of an individual pesticide compound used in agriculture and may reflect exposure via multiple pathways. Food is an important pathway of pesticide exposure for children and this study does not distinguish between pesticide exposure via the diet or via the ambient environment. Therefore, DAP levels observed in this study represent integrated levels of exposure from several environmental sources. Dietary exposure to OPs may also have reduced our ability to demonstrate differences between AG and Non-Ag children.

Classification of chronic pesticide exposure by parental occupation also has limitations. This classification makes the assumption that a parent working in agriculture is exposed to OP pesticides in the workplace and the child is indirectly. The study was designed to create a sample of children with chronic pesticide exposure by requiring that parents work in agriculture at the time of the study and had worked in agriculture for at least 3 years prior. In addition, the parents of non-AG children were required to not have a history of working in agriculture within the past 3 years.

Lambert et al. (2005) demonstrated that substantial variation in OP metabolite levels across communities hosting differing agricultural industries. This study did not divide up the group of AG children by agricultural industry. Lambert et al. (2005) suggests that failure to account for difference between communities may result in exposure misclassification.

Residual and Unmeasured Confounding

In this study, residual confounding may results from either improper definition of the categories of the confounding variable or an imperfect measure of the confounder. Unmeasured confounding arises from other important confounders that were not accounted for in the model.

Categories of continuous variables in this analysis included age groups (< 6 years and ≥ 6 years) and maternal education groups (no high school, some high school, and high school graduate). These categories were defined based on differences in potential exposure to pesticides and performance on neurobehavioral tests. Residual confounding is not likely within age groups and maternal educational groups.

The variable for maternal education was used as a proxy for socioeconomic status and this variable is not a complete representation of socioeconomic status. It is possible that socioeconomic differences may exist between the two groups within the maternal education categories. It has been shown that individuals of higher socioeconomic status eat more fresh fruit and vegetables than individuals of poorer environments (Bhargava and Hays 2004) and children of higher socioecomic status may receive more education and perform better on neurobehavioral tests. Therefore, socio economic status is a potential confounder not completely controlled for in this analysis.

Additionally, this analysis did not include information on computer access. Previous research by Li and Atkins (2004) found preschool children enrolled in Head Start who had access to a computer performed better on measures of school readiness and cognitive development.

Chance

The interactions presented in this thesis analysis should be carefully interpreted. All five DAP metabolites were detected 5 children of 181 children included in this study. As a result, differences in test performance for children with greater than 4 metabolites detected were not statistically significant. However, the study was able to demonstrate statistically significant differences in test performance between children exposed to more pesticides. Given the small sample size, a Type II Error—failing to identify an association that exists in the population—is possible, though unlikely. Rohlman and colleagues (2005) conducted an analysis with a subset of the population included in this study, and was able to demonstrate significant differences with a smaller sample size.

Strengths

Careful thought and consideration went into the study design to reduce the role of bias, confounding and chance. The following bullets summarize the strengths of this study:

- Neurobehavioral tests were carefully administered and quality assurance/quality control measures were implemented to ensure high quality data.
- In order to eliminate potential misclassification bias, exposure data were collected at multiple time points.

- Urine samples are an objective marker of exposure and therefore, reduce the potential for information bias in exposure classification.
- By study design, the children included in this study were a homogenous study population of Latino children age 4-11 yrs. A homogonous study population reduces the possibility of confounders such as age and ethnicity.
- This study included children from a relatively large geographic region with diverse agriculture. As a result, pesticide exposure was heterogeneous across communities. By combining children from different agricultural regions in Oregon, results are more generalizable to children in other Oregon communities and potential other communities across the US.
- The data were collected following CBPR principles and included a community involvement component on all aspects of the project.
- The CBPR approach also allowed researcher to reach a minority population and educate families on the health concerns of pesticide exposure. These data were collected to inform public health action and have resulted in a valuable contribution to a scientific knowledge base and toward improving the health of a vulnerable population.

Public Health Implications

Proper management, use, and disposal of pesticides are important public health issues in Oregon and around the world. Although trends in pesticide use in the US are downward, the global use of pesticides is on the rise, with about half of the increase in pesticide use occurring in developing countries (WHO, 1990). Children in other countries will continue to be exposed to OP pesticides and food imported from other countries to the US will continue to contain pesticide residues. This analysis examines the effects of relatively low-level exposure to OP pesticides in the US and represents a "best case scenario." Results have international public health implications where pesticide use in not regulated and exposure is at higher levels.

In Oregon, public health action should continue to aim to prevent pesticide exposure in the working the living environment. Within the workplace action may include educational programs to reduce the likelihood of the carry-home effect by promoting basic hygiene and safe work practices. Non-agricultural workplaces may reduce pesticide exposure by adopting of integrated pest management programs. Within the living environment individual action can be taken to reduce pesticide exposure, including: reducing pesticide use in the home, improving cleaning practices, and consuming organic foods.

Public health regulators and academic researchers should continue to gain knowledge on the effectiveness of community-based interventions in agricultural communities. Tracking systems such as The Oregon Department of Agriculture Pesticide Use Reporting System, are useful tools in helping to understand when, where and what types and quantities of pesticides are being used in Oregon. This information would be used to monitor potential environmental exposure hazards and prevent public health problems.

Conclusion

The results of this analysis have demonstrated a correlation between pesticide exposure and certain neurobehavioral outcomes, and similar findings have been supported in other studies. Many factors may contribute to the poor health of the migrant farm worker community in Oregon; however, exposure to pesticides is one factor that can be controlled. This analysis has limitations, and in light of these limitations the results continue support the developing body of knowledge that has demonstrated agriculture workers are exposed to more pesticides than non-agriculture workers. Neurobehavioral effects are just one negative health effect of pesticide exposure, there are many other health effects currently under investigation and possibly not yet considered. Precaution should be taken to protect the health of the families of this important population of workers in the US and around the world.

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Appendix A:
DAP Metabolite Linear Regression Model Results

Table A-1. Relationship between DMP concentration and agricultural status adjusted for gender, age, maternal education

	β (SE)	95% CI	p-value
Agricultural Status			
Non-Ag	Reference		
Ag	6.39 (3.29)	-0.12, 12.89	0.05
Gender			
Male	Reference		
Female	4.21 (2.76)	-1.25, 9.67	0.13
Age group			
<6 years	Reference		
≥6yrs	-3.70 (2.85)	-9.32, 1.94	0.20
Maternal education			
<9 yrs	Reference		
9-12 yrs	-3.37 (3.69)	-10.65, 3.92	0.36
>12 yrs	-3.22 (4.05)	-11.23, 4.79	0.43

Table A-2. Relationship between DEP concentration and agricultural status adjusted for gender, age, and maternal education

	β (SE)	95% CI	p-value
Agricultural Status			
Non-Ag	Reference		
Ag	1.26 (1.12)	-0.95, 3.47	0.26
Gender			
Male	Reference		
Female	-0.05 (0.94)	-1.90, 1.81	0.96
Age group			
<6 years	Reference		
≥6yrs	-0.81 (0.97)	-2.72, 1.11	0.41
Maternal education			
<9 yrs	Reference		
9-12 yrs	0.24 (1.25)	-2.23, 2.72	0.85
>12 yrs	-0.69 (1.38)	-3.42, 2.03	0.62

Table A-3. Relationship between DMTP concentration and agricultural status adjusted for gender, age, and maternal education

	β (SE)	95% CI	p-value
Agricultural Status			
Non-Ag	Reference		
Ag	-21.09 (14.67)	-50.08, 7.90	0.15
Gender			
Male	Reference		
Female	14.67 (12.31)	-9.67, 39.00	0.24
Age group			
<6 years	Reference		
≥6yrs	-31.16 (12.70)	-56.27, -6.06	0.02
Maternal education			
<9 yrs	Reference		
9-12 yrs	-30.97 (16.44)	-63.46, 1.52	0.06
>12 yrs	-17.26 (18.06)	-52.96, 18.44	0.34

Table A-4. Relationship between DMDTP concentration and agricultural status adjusted for gender, age, and maternal education

	β (SE)	95% CI	p-value
Agricultural Status			
Non-Ag	Reference		
Ag	4.02 (3.16)	-2.23, 10.26	0.21
Gender			
Male	Reference		
Female	2.41 (2.65)	-2.83, 7.66	0.36
Age group			
<6 years	Reference		
≥6yrs	-2.05 (2.74)	-7.46, 3.36	0.46
Maternal education			
<9 yrs	Reference		
9-12 yrs	-1.07 (3.54)	-8.07, 5.93	0.76
>12 yrs	-4.07 (3.89)	-11.76, 3.62	0.30

Table A-5. Relationship between DETP concentration and agricultural status adjusted for gender, age, and maternal education

	β (SE)	95% CI	p-value
Agricultural Status			
Non-Ag	Reference		
Ag	-5.46 (15.36)	-35.80, 24.91	0.72
Gender			
Male	Reference		
Female	-10.35 (12.89)	-35.83, 15.13	0.42
Age group			
<6 years	Reference		
≥6yrs	-19.61 (13.30)	-45.90, 6.68	0.14
Maternal education			
<9 yrs	Reference		
9-12 yrs	-18.84 (17.21)	-52.86, 15.18	0.28
>12 yrs	-22.14 (18.91)	-59.52, 15.24	0.24

Appendix B:
Neurobehavioral Test Linear Regression Model Results

Table B-1. Relationship between Finger Tapping: Number of Taps Left performance and the number of DAPs Detected adjusted for agriculture status, gender, age, and maternal education (Adj. R^2 = 49 %)

	β (SE)	95% CI	p-value
# of DAPs	2.24 (0.87)	0.51, 3.96	0.01
# DAPs x Gender	-2.81 (1.21)	-5.19, -0.43	0.02
# DAPs x Age Group	0.37 (0.21)	-0.04, -0.78	0.08
Gender			
Male	Reference		
Female	-1.07 (2.17)	-5.35, 3.21	0.62
Age group			
<6 years	Reference		
≥6yrs	14.37	10.17, 18.57	0.00
Maternal education			
<9 yrs	Reference		
9-12 yrs	1.81 (1.88)	-1.90, 5.53	0.34
>12 yrs	3.00 (2.04)	-1.03, 7.04	0.14

Table B-2. Relationship between Continuous Performance Hits performance and the number of DAPs Detected adjusted for agriculture status, gender, age, and maternal education (Adj. R^2 = 4 %)

	β (SE)	95% CI	p-value
# of DAPs	1.58 (0.70)	0.20, 2.97	0.03
Status			
Non-AG	Reference		
AG	3.73 (1.47)	0.82, 6.64	0.01
Status x # DAPs	-2.17 (0.83)	-3.82, -0.53	0.01
Gender			
Male	Reference		
Female	-0.57 (1.01)	-2.57, 1.42	0.569
Age group			
<6 years	Reference		
≥6yrs	1.73 (0.99)	-0.22, 3.68	0.082
Maternal education			
<9 yrs	Reference		
9-12 yrs	-1.13 (1.27)	-3.65, 1.39	0.38
>12 yrs	1.01 (1.48)	-1.93, 3.95	0.50

Table B-3. Relationship between Continuous Performance: Misses performance and the number of DAPs Detected adjusted for agriculture status, gender, age, and maternal education (Adj. R^2 = 4 %)

	β (SE)	95% CI	p-value
# of DAPs	-1.53 (0.68)	-2.88, -0.18	0.03
Status			
Non-AG	Reference		
AG	-3.73 (1.43)	-6.56, -0.89	0.01
Status x # DAPs	2.13 (0.81)	0.53-3.73	0.01
Gender			
Male	Reference		
Female	0.73 (0.98)	-1.21-2.67	0.46
Age group			
<6 years	Reference		
≥6yrs	-1.78 (0.96)	-3.68-0.12	0.07
Maternal education			
<9 yrs	Reference		
9-12 yrs	1.04 (1.24)	-1.41 – 3.50	0.40
>12 yrs	-1.17 (1.44)	-4.04 – 1.68	0.42

Table B-4. Relationship between Continuous Performance: Percent Corrected Hits performance and the number of DAPs Detected adjusted for agriculture status, gender, age, and maternal education (Adj. R^2 = 4 %)

	β (SE)	95% CI	p-value
# of DAPs	0.05 (0.02)	0.01, 0.10	0.01
Status			
Non-AG	Reference		
AG	0.13 (0.05)	0.03, 0.22	0.01
Status x # DAPs	-0.07 (0.03)	-0.13, -0.02	0.01
Gender			
Male	Reference		
Female	-0.02 (0.03)	-0.09, 0.04	0.49
Age group			
<6 years	Reference		
≥6yrs	0.06 (0.03)	-0.01, 0.12	0.07
Maternal education			
<9 yrs	Reference		
9-12 yrs	-0.03 (0.04)	-0.12, 0.05	0.39
>12 yrs	0.04 (0.05)	-0.06, 0.13	0.44

Table B-5. Relationship between Divided Attention Words/Language: Right Hand Taps performance and the number of DAPs Detected adjusted for agriculture status, gender, age, and maternal education (Adj. R^2 = 38 %)

	β (SE)	95% CI	p-value
# of DAPs	2.24 (0.69)	0.88, 3.60	0.001
Gender			
Male	Reference		
Female	-5.47 (1.69)	-8.80, 2.14	0.00
Age group			
<6 years	Reference		
≥6yrs	14.43 (1.70)	11.07, 17.78	0.00
Maternal education			
<9 yrs	Reference		
9-12 yrs	0.77 (2.13)	-3.46, 4.99	0.72
>12 yrs	3.36 (2.36)	-1.30, 8.02	0.16

Table B-6. Relationship between Divided Attention Words/Language: Left Hand Taps performance and the number of DAPs Detected adjusted for agriculture status, gender, age, and maternal education (Adj. R^2 = 40 %)

	β (SE)	95% CI	p-value
# of DAPs	2.95 (0.74)	1.48, 4.42	0.00
Status			
Non-AG	Reference		
AG	-3.57 (1.65)	-6.83, -0.30	0.03
Gender x # DAPs	-3.00 (1.13)	-5.23, 0.78	0.01
Gender			
Male	Reference		
Female	-0.85	-4.78, 3.08	0.67
Age group			
<6 years	Reference		
≥6yrs	11.38 (1.38)	8.66, 14.12	0.00
Maternal education			
<9 yrs	Reference		
9-12 yrs	1.19 (1.77)	-2.32, 4.70	0.50
>12 yrs	1.54 (2.08)	-2.57, 5.65	0.46

Table B-7. Relationship between Object Memory: Immediate Recall performance and the number of DAPs Detected adjusted for agriculture status, gender, age, and maternal education (Adj. $R^2 = 20$ %)

	β (SE)	95% CI	p-value
Status			
Non-AG	Reference		
AG	0.81 (0.38)	0.06, 1.56	0.034
Gender			
Male	Reference		
Female	0.23 (0.32)	-0.40, 0.86	0.48
Age group			
<6 years	Reference		
≥6yrs	2.01 (0.33)	1.36, 2.45	0.00
Maternal education			
<9 yrs	Reference		
9-12 yrs	0.30 (0.42)	-1.12, 0.52	0.47
>12 yrs	0.03 (0.49)	-0.92, 0.99	0.95

Table B-8. Relationship between Object Memory: Recognition performance and the number of DAPs Detected adjusted for agriculture status, gender, age, and maternal education (Adj. $R^2 = 10$ %)

	β (SE)	95% CI	p-value
# DAPs	0.53 (0.34)	-0.14-1.20	0.12
# DAPs x Gender	-1.39 (0.52)	-2.41, -0.36	0.01
Gender			
Male	Reference		
Female	1.09 (0.93)	-1.46, 2.21	0.69
Age group			
<6 years	Reference		
≥6yrs	2.19 (0.63)	0.94, 3.43	0.00
Maternal education			
<9 yrs	Reference		
9-12 yrs	-0.52 (0.77)	-2.05, 1.01	0.50
>12 yrs	0.37 (0.93)	-1.46, 2.21	0.69

Table B-9. Relationship between Purdue Pegboard: Number of Pegs Right performance and the number of DAPs Detected adjusted for agriculture status, gender, age, and maternal education (Adj. $R^2 = 58$ %)

	β (SE)	95% CI	p-value
# of DAPs	0.55 (0.22)	0.10, 0.99	0.02
Status			
Non-AG	Reference		
AG	0.89 (0.47)	-0.04, 1.82	0.06
Status x # DAPs	-0.50 (0.26)	-1.02, 0.1	0.06
Gender			
Male	Reference		
Female	0.16 (0.29)	-0.42, 0.74	0.59
Age group			
<6 years	Reference		
≥6yrs	4.38 (0.30)	3.79, 4.97	0.00
Maternal education			
<9 yrs	Reference		
9-12 yrs	0.25 (0.38)	-0.50, 1.01	0.51
>12 yrs	0.35 (0.44)	-0.52, 1.23	0.42

Table B-10. Relationship between Purdue Pegboard: Number of Pegs Left performance and the number of DAPs Detected adjusted for agriculture status, gender, age, and maternal education (Adj. $R^2 = 52\ \%$)

	β (SE)	95% CI	p-value
# of DAPs	0.23 (0.12)	-0.01, 0.48	0.06
Gender			
Male	Reference		
Female	0.13 (0.30)	-0.46, 0.73	0.66
Age group			
<6 years	Reference		
≥6yrs	3.86 (0.31)	3.25, 4.46	0.00
Maternal education			
<9 yrs	Reference		
9-12 yrs	-0.1 (0.38)	-0.76-0.74	0.98
>12 yrs	-0.36 (0.42)	-1.20-0.48	0.40

Table B-11. Relationship between Purdue Pegboard: Number of Pegs Both performance and the number of DAPs Detected adjusted for agriculture status, gender, age, and maternal education (Adj. $R^2 = 52\ \%$)

	β (SE)	95% CI	p-value
# of DAPs	0.16 (0.11)	-0.05, 0.38	0.14
Gender			
Male	Reference		
Female	0.17 (0.26)	-0.36, 0.69	0.53
Age group			
<6 years	Reference		
≥6yrs	3.38 (0.27)	2.85, 3.91	0.00
Maternal education			
<9 yrs	Reference		
9-12 yrs	-0.13 (0.33)	-0.79, 0.54	0.71
>12 yrs	0.27 (0.37)	-0.46, 1.00	0.47

Table B-12. Relationship between Visual Motor Integration performance and the number of DAPs Detected adjusted for agriculture status, gender, age, and maternal education (Adj. $R^2 = 40\ \%$)

	β (SE)	95% CI	p-value
# of DAPs	0.30 (0.17)	-0.04, 0.64	0.083
Gender			
Male	Reference		
Female	-0.58 (0.42)	-1.42, 0.25	0.17
Age group			
<6 years	Reference		
≥6yrs	5.03 (0.43)	4.18, 5.88	0.00
Maternal education			
<9 yrs	Reference		
9-12 yrs	-0.61 (0.53)	-1.66, 0.44	0.26
>12 yrs	0.78 (0.58)	-0.37, 1.93	0.18