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Organophosphate Pesticide Exposure and Neurobehavioral Performance in Agricultural and Non-Agricultural Hispanic Workers

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List of abbreviations with definitions:

- (AZM) Azinphos-methyl
- (BARS) Behavioral Assessment and Research System
- (DAP) Dialkyl phosphate metabolites
- (DEP) Diethylphosphate
- (DETP) Diethylthiophosphate
- (DMDTP) Dimethyldithiophosphate
- (DMP) Dimethylphosphate
- (DMTP) Dimethylthiophosphate
- (GC/MS) Gas Chromatography/Mass Spectrometry
- (GWPL) Groundwater Protection List
- (LOD) Limits of detection
- (NB) Neurobehavioral
- (OCDC) Oregon Child Development Coalition

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Abstract

Our understanding of the health risks of farmworkers exposed to pesticides in their work and home environment is rapidly increasing, although studies designed to examine the possible neurobehavioral effects of low level chronic pesticide exposure are limited. We measured dialkyl phosphate urinary metabolite levels, collected environmental dust samples from a subset of homes, obtained information on work practices and conducted neurobehavioral tests on a sample of farmworkers in Oregon. Significant correlations between urinary methyl metabolite levels and total methyl organophosphate (azinphos-methyl, phosmet, malathion) house dust levels were observed. We found the neurobehavioral performance of Hispanic immigrant farmworkers to be lower than that observed in a non-agricultural Hispanic immigrant population, and within the agricultural workers there was a positive correlation between urinary organophosphate metabolite levels and poorer performance on some neurobehavioral tests. These findings add to an increasing body of evidence of the association between low levels of pesticide exposure and deficits in neurobehavioral performance.

Introduction

In recent years there has been increasing concern regarding the widespread use of pesticides in agricultural communities and potential impacts on public health. In the 1990s in the United States some 2.5 million to 5.0 million agricultural workers were exposed to organophosphate insecticides (Das et al. 2001). Scientific field investigations have focused on delineating the extent of exposure and potential health effects in agricultural and non-agricultural communities. Detectable levels of pesticides have been reported in home dust, primarily in families residing in agricultural areas (Arcury et al. in press; Bradman et al. 1997; McCauley et al. 2001; Quandt et al. 2004; Simcox et al. 1995). Bradman et al. (1997) found that diazinon and chlorpyrifos house dust concentrations tended to be higher among farmworkers than non-farmworkers. Others have reported higher levels of pesticides in house dust in homes that are located closer to fields (Quandt et al. 2004) and in housing with larger numbers of farmworkers (Azaroff 1999; Lu et al. 2000; McCauley et al. 2001). After-work hygiene practices, such as leaving work boots outside, and changing promptly from work clothes, have also been found to affect pesticide levels in the homes of farmworkers (McCauley et al. 2003).

Studies have also documented the presence of biological markers of pesticide exposure in adults and children in agricultural communities (Arcury and Quandt 2003; Azaroff 1999; Loewenherz et al.1997; O'Rourke et al. 2000) and differences among levels of exposure in residents of agriculture and non-agricultural communities. While the association between acute exposure to pesticides and neurotoxic effects is well known (Lotti 2000), the potential effects of chronic low-level exposure are less well established (Alavanja et al. 2004).

Neurobehavioral test batteries have frequently been used to examine neurobehavioral effects of acute pesticide exposure in adult working populations. Individuals with histories of toxic exposures to organophosphates have shown a consistent pattern of deficits on measures of motor speed and coordination, sustained attention and information processing speed (Reidy et al. 1992; Rosenstock et al. 1991; Savage et al.1988; Steenland et al.1994; Wesseling et al. 2002). Fewer studies have examined the effect of long-term, low-level exposure to pesticides on nervous system functioning, but neurobehavioral changes have been reported in sheep farmers (Stephens et al. 1995), greenhouse workers (Bazylewicz et al.1999), tree fruit workers (Fiedler et al. 1997), and farmworkers in Florida (Kamel et al. 2003). These studies have found deficits in measures of sustained attention, information processing, and motor speed and coordination. An examination of a group of cotton pesticides applicators in Egypt presumed to have high exposures, found a broad range of deficits including visual motor speed, verbal abstraction, attention, and memory (Farahat et al. 2003).

While these studies represent increasing knowledge regarding the association between pesticide exposure and neurological health endpoints, there have been few studies that report the association between environmental exposures, biomarkers of exposure, and neurological performance. We conducted an investigation of migrant farmworkers in Oregon and included measures of environmental exposure, biomarkers of exposure and neurobehavioral performance. In this study it was hypothesized that

1) significant correlations would be found between the amount of organophosphate residues in house dust and the levels of organophosphate metabolites in urines of adult farmworkers living in an agricultural community;
2) the neurobehavioral performance of Hispanic immigrant farmworkers exposed to organophosphates would be lower than that observed in a non-agricultural Hispanic

immigrant population when controlled for demographic factors such as age and education; and

3) within the agricultural workers there would be a positive correlation between urinary organophosphate metabolite levels and poorer neurobehavioral performance.

Methods

Target communities The agricultural community, Hood River, is a productive and long-established agricultural community primarily producing pears and apples and located along the Columbia Gorge, approximately 100 km east of Portland, OR. The farmworker population in Hood River tends to consist of newly arrived and more permanent Hispanic residents who live in cabins, trailers, single and multi-family homes or apartments that are located in or alongside orchards. Harvesting of tree fruit begins in August and extends through October. The study was conducted as a partnership with the Oregon Child Development Coalition (OCDC), which is the grantee for Oregon Migrant HeadStart Program. Ninety-six farmworkers were recruited by community members of the Migrant Head Start program in Hood River. All attendees at parent meetings at Migrant Head Start who had a child enrolled in a migrant Head Start program and were currently working in the orchards, fields and nurseries, were invited to participate. Participants ranged in age from 20-52 years old and were all originally from Mexico. Some of the parents first arrived in the U.S, in 1970 and some parents had just arrived for the first time in 1998. After the families were recruited they were scheduled for a home visit when questionnaires were administered and dust samples collected.

To compare performance on neurobehavioral tests we recruited immigrant workers from Newport City, a tourist coastal area with little agriculture. The Hispanic workforce in Lincoln

County consists of immigrant workers who are primarily employed by the local hotels and tourist industry. Most of these individuals came to Oregon 6-8 years earlier after they were solicited in Mexico to work in the Oregon fish canning industry. When the canning business declined these workers remained in Newport City to work in hotels and restaurants. They were recruited for this study by a community member with the support and partnership of the Hispanic community organizations, *Centro de Ayuda* and *Un Paso Adelante*. The individuals were recruited one-on-one through word-of-mouth, community contacts, and at neighborhood grocery stores. Workers were eligible to participate in the study if they had not worked in agriculture during the previous three months, including nurseries, farms and fruit packing plants, were 18-50 years old, had not attended school in the U.S. other than ESL classes (English as a Second Language), did not use a computer at work, and had never had an acute illness associated with pesticide exposure.

All biological samples and neurobehavioral assessments of both farmworkers from Hood River and the control group from Lincoln City were conducted in the evenings after their workday. Participants were paid an incentive for participating in this study. The study protocol and procedures for informed consent were reviewed and approved by the OHSU University Institutional Review Board (Protocol #4216) and complied with all applicable requirements of the U.S.A. regulations.

Data collection Spot-urine samples for pesticide metabolite analysis were collected from farmworkers once during the summer and again in the fall. Samples were collected from each farmworker at the Migrant Head Start Center in the evening after work just prior to taking the neurobehavioral tests. Samples were labeled, and transferred on ice to the OHSU analytical laboratory. Urine specimens were adjusted to pH 3.0, aliquoted into test tubes and all urine was stored at $-20\ ^{o}C$ until extraction and analysis.

House dust samples were collected from a sub-sample of 26 farmworker's homes during the same week as collection of the first urine sample. Azinphos-methyl (trade name Guthion, CAS No. 86-50-0), chlorpyrifos (CAS No. 2921-88-2), and phosmet (trade name Imidan, CAS No. 732-11-6) are used to control orchard pests such as coddling moth and are applied two to four times from May through August in the Hood River community. The period of time when home dust samples were collected was within a week of reported organophosphate applications at nearby farms not participating in the study in the middle of the summer growing season. Dust samples were collected using a high volume, small surface sampler (HVS3) as described by Lewis (1994) and Simcox (1995). All samples were collected from carpeted areas in the most commonly used play area for their children and living area for adults. All samples were collected in Teflon (E.I. Dupont Company, Wilmington, DE) bottles by vacuuming a measured area on a rug or carpet designed to collect an approximate 5g sample. Samples were transported to the lab in a refrigerated cooler and stored below $-20\ ^{o}C$ prior to analysis.

Both the farmworkers and control participants were tested in the evenings after work. Control participants were tested in the spring and the farmworker participants were tested in the summer and fall. Although testing was conducted in different locations, a uniform testing environment was created in both settings for the neurobehavioral testing. Testing stations were set up by using panel dividers to partition tables into different stations. Each station contained a computer, response unit and headphones. Instructions on how to complete the computerized tests were given in Spanish. Four to six participants were tested at one time in air-conditioned meeting rooms. Neurobehavioral (NB) tests were selected from the Behavioral Assessment and Research System (BARS). BARS is a computerized test system that employs both written and spoken instructions (both via computer; Rohlman et al. 2003). To minimize the adverse impact of working on an unfamiliar device such as a computer keyboard, a durable response unit with nine buttons is placed over the keyboard (pictured in Anger et al. 1996). The BARS test instructions have been translated into Spanish, recorded and digitized. Instructions were written in Spanish on the screen and also delivered simultaneously through headphones. The eight BARS tests include measures of psychomotor functioning (Finger Tapping, Simple Reaction Time, and Progressive Ratio) and measures of cognitive functioning (Symbol-Digit, Digit Span, Selective Attention, Serial Digit Learning, and Continuous Performance). More information about these tests can be found at http://home.comcast.net/~neta-lo/BARS.html.

Laboratory analysis Dust samples were put through a sieve, extracted with organic solvents, and cleaned up using gel permeation chromatography and analyzed on a Hewlett Packer Model 5890 Gas Chromatograph equipped with a pulse flame photometric detector (OI Analytical). The organophosphates, azinphos-methyl, diazinon, chlorpyrifos, malathion, methyl parathion and phosmet) were confirmed with gas chromatography/mass spectrometry (GC/MS) mass selective detector using a single ion monitoring mode. Specific methods for sample extraction and sample cleanup, involving filtration and GPC column cleanup and GC analysis, have been previously described (Moate et al. 2002). The limits of detection (LOD) for the six organophosphates were 0.01 μg/gm for diazinon, malathion, chlorpyrifos and methyl parathion, and 0.10 μg/gm for azinphos-methyl and phosmet.

Urine was analyzed for five dialkyl phosphate (DAP) metabolites: dimethylphosphate (DMP), diethylphosphate (DEP), dimethylthiophosphate (DMTP), dielthythiophosphate (DETP), and dimethyldithiophosphate (DMDTP). Urine samples were prepared for GC analysis 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-pentafluorobenzylbromide to convert phosphate acids to esters. Extracted samples were analyzed on a gas chromatograph (Hewlett-Packard Model 5890, Palo Alto, CA) equipped with a pulsed-flame photometric detector (OI Analytical, College Station, TX). The limit of detection (LOD) for each of the metabolites was calculated from the instrument response factor corresponding to a concentration having a peak area three times the baseline noise (blank signal). The limits of detection for the five metabolites were 4.0 ng/ml (0.032 µmoles/L) DMP, 2.0 ng/ml (0.013 µmoles/L) DEP, 2.2 ng/ml (0.015 µmoles/L) DMTP, 1.6 ng/ml (0.010 µmoles/L) DMDTP, and 1.6 ng/ml (0.0095 µmoles/L) DETP. The average extraction efficiencies (of the five metabolites were, respectively: 45, 84, 97, 96, and 93 percent. Urine samples were also analyzed for creatinine concentration. Creatinine concentrations (mg/dL) were determined by the modified Jaffe reaction (Creatinine Procedure No. 555, Sigma Diagnostics, Dorset, UK Sigma Procedure 555, St. Louis, MO).

Quality control/Quality assurance Quality control data generated for each set of urine samples were used to provide an overall assessment of precision, accuracy and overall reliability of the method. Spike sample recoveries and urine blank analysis were conducted for every set of 12 samples. Urine samples known to contain low levels of DAP were used for blanks and for spike recoveries. Urine samples were spiked with DAP reference standards varying in concentration from 2 to 50 ng/ml.

Data analysis The distribution of creatinine was examined and urine samples less than the 5th percentile (26.45 mg/dL) or greater than the 95th percentile (235.5 mg/dL) were excluded from further analysis due to concerns of hydration state and metabolic disorders (Loewenherz et al.1997; Lu et al. 2001). The primary organophosphates applied during the spring and summer season in the agricultural regions under study were azinphos methyl and phosmet, both of which break down into the methyl dialkylphosphate metabolites (DMTP and DMDTP). Therefore, for urine samples, molar equivalent concentrations of the DMTP and DMDTP metabolites were summed to create a measure of thiomethyl DAP concentration. Non-detectable levels of urinary metabolites were replaced by one-half the appropriate limit of detection (LOD) prior to taking the sum.

For house dust samples, residues associated with azinphos-methyl, phosmet, and malathion (the most common agricultural organophosphated used in the study region) were added together to form a summary measure of pesticides in the house dust. Each of these pesticides metabolize into the thiomethyl DAPs. Non-detectable levels of dust residues were replaced by one-half the appropriate limit of detection (LOD) prior to taking the sum.

The association between methyl phosphates in house dust and thiomethyl concentrations in urine was evaluated using Spearman's correlation. The difference in urinary thiomethyl metabolites between the first testing session (T1) and the second testing session (T2) was evaluated with a Wilcoxon signed-rank test. This test suggested thiomethyl metabolites from T1 and T2 could be combined for subsequent analyses. Subjects with valid creatinine levels from both sessions had their metabolite levels averaged over the two sessions; subjects with a valid creatinine level from only one session contributed metabolite data from only that session. The partial correlation (Rao 1973) was computed to examine the association between neurobehavioral test performance from T1 and the averaged thiomethyl metabolite levels after accounting for the effects of sex, age and education in the subject's country of origin (age and education were treated as continuous variables; sex was a two-level factor). This analysis was conducted for subjects having a valid creatinine level during at least one of the two testing sessions. Differences on each neurobehavioral test between agricultural and non-agricultural communities were assessed using multiple linear regression models involving age, sex, years of education in the subject's country of origin, and an indicator for agricultural status. Three interactions between agricultural status and each of the other predictors were also included in the initial model and simultaneously tested for significance using a partial F-test (Netter et al. 1989). If the test was significant ($p<0.10$) then each interaction was separately examined and retained in the model if individually significant (again at the $p<0.10$ level). Adjusted values reported from the regression model reflect the mean score on each NB test for a 25 year old subject with six years of education in their country of origin.

To increase the power to detect effects of exposure between the agricultural and comparison population a summary index of overall NB performance was derived from 11 of the 16 NB test items (digit span forward, digit span reverse, progressive ratio, reaction time, selective attention inter-stimulus interval, serial digit learning, symbol-digit, preferred hand finger tapping, non-preferred hand finger tapping, alternating hand finger tapping, and continuous performance percent hits).The items for the summary index were chosen to provide an equal representation of all the multiple measures in the test battery and were chosen prior to identification of the individual items that were statistically different between the two comparison groups. Measurements for each test were first standardized by subtracting the mean and dividing the difference by the sample standard deviation. Tests involving latency measures had the signs of the standardized measurements reversed to provide consistency with the other measures (higher numbers indicating better performance, lower numbers weaker performance). The summary index was computed as each subject's average standardized score from the test items divided by the standard error of the mean. The summary index was similarly analyzed to determine whether significant partial correlations existed with thiomethyl metabolites or if significant differences existed between the two agricultural communities.

All *p*-values are two-sided unless otherwise indicated. One sided *p*-values were used in cases where the means or correlations were anticipated to follow a pre-chosen trend. All analyses were performed with R version 1.9.1 (R Development Core Team 2004).

Results

Ninety-nine farmworkers were attending the parent meeting at Head Start and were approached for study participation, with only three declining to participate. Fifty-five controls were recruited for the study, but 10 were excluded because they were either working in landscaping or tree planting (forestry), had no formal education in Mexico or the U.S., or were not available during scheduled testing times. All farmworkers were immigrants from Mexico and the controls were primarily from Mexico (two participants were from Guatemala and one unknown). There was no significant difference in the ages of the two groups (farmworkers 20-52 years old (mean 29.7, SD = 6.89), controls 19-48 years old (mean 27.8, SD=6.19) The control group averaged 1.1 years more education than the farmworkers ($p = 0.04$; 95% CI: 0.026 to 2.2 years more). The percentage of males in the two groups was not significantly different ($p = 0.33$). The mean number of years since first arrival in the U.S. was 9.8 years for farmworkers and 7.3 years for controls.

Pesticide residue in house dust Our pesticide data included carpet dust samples from 26 farmworker's homes and descriptions of the six organophosphates for which we analyzed are reported in Table 1. At least one of the six organophosphates was detected in each of the homes. Phosmet, with a median detected concentration of 4.40 μg/g, was detected in 25 of the 26 homes

(96%). Azinphos-methyl (AZM) was detected in 18 of the 26 homes (69%) but had a higher detected median concentration (5.30 μg/g). Both of these organophosphates are not registered for residential use and spray records from local growers in the area reported orchard application of phosmet and AZM several (2–4) times from May until August. The organophosphates, chlorpyrifos, parathion, malathion and diazinon were detected at frequencies between 62 and 92% but at median detectable concentrations several times lower than found for AZM or phosmet.

[Insert Table 1 about here]

Urinary metabolite levels The two testing sessions with farmworkers provided a total of 172 urine samples (93 samples at Time 1 (T1) and 79 at Time 2 (T2)). Analysis of the urinary metabolites was performed on all samples, but two samples were of insufficient volume for subsequent creatinine analysis. The distribution of creatinine levels in the remaining 170 samples was examined and urine samples less than the 5th percentile (26.45 mg/dL) or greater than the 95th percentile (235.5 mg/dL) were excluded from further analysis due to concerns of hydration state and metabolic disorders. This restriction reduced to the number of valid urine samples to 84 and 68, respectively, for the first and second testing sessions; 88 subjects had valid urine samples for at least one of the two testing sessions.

DMTP was the most commonly quantified organophosphate metabolite (Table 2). The percentage of the complete sample above the detection limit was 97% and 100%, respectively, for the first (T1) and second sample periods (T2). When computed for samples with valid creatinine levels, the median concentration of the combined thiomethyl metabolites (DMTP, DMDTP) was 0.43 μmoles/L in the summer (Time 1, $n = 84$) and 0.48 μmoles/L in the fall

(Time 2, $n = 68$); the median increases to 0.56 µmoles/L when data from the two time periods were averaged together and the sample broadened to include subjects with at least one valid creatinine measurement (from Time 1 or Time 2).

[Insert Table 2 about here]

No significant differences were found between the median concentrations of thiomethyl metabolites from the two periods ($p > 0.20$ for both DMTP and DMDTP; Wilcoxon signed-rank test). Males tended to have higher levels of DMTP and combined thiomethyl metabolites at both time points compared to female farmworkers.

Correlation of home dust samples and urinary metabolite levels Twenty-three of the 26 samples of pesticide residues found in carpet dust could be paired with the combined molar concentration of thiomethyls (DMTP and DMDTP) from valid urine samples. A moderate but significant positive correlation existed between these 23 pairs of methyl pesticides (sum of AZM, phosmet and malathion) measured in carpet dust and the combined molar concentrations of the thiomethyl metabolites (DMTP+DMDTP; µmoles/L) (See Figure 1). The impact of the three upper-most points observed in Figure 1 is reduced when summarized using Spearman's correlation ($r_s = 0.47$, 1-sided p=0.013).

[Insert Figure 1 about here]

Correlation between neurobehavioral performance and urinary metabolite levels Ninety-two farmworkers (51% male) and 45 controls (60% male) completed neurobehavioral tests. Neurobehavioral performance was compared to the combined thiomethyl metabolites

(DMTP+DMDTP) averaged across the two urine samples). After adjusting for age, gender, and years of education, poorer performance on five neurobehavioral tests was associated with higher levels of the average combined thiomethyl metabolites: Selective Attention latency, Symbol-Digit latency, preferred hand Finger Tapping, Alternating Hand Finger Tapping (or Finger Tapping alternate hands), and Continuous Performance hit latency.

[Insert Table 3 about here]

Comparison of neurobehavioral performance between farmworkers and controls Overall, non-agricultural controls performed better on 12 out of 16 NB measurements compared to ninety-two farmworkers (Table 4). Multiple linear regression was used to compare performance on the neurobehavioral tests between the agriculture and non-agriculture groups while controlling for age, years of education in country of origin, and gender. Interactions between these three covariates and employment in agriculture may have also been included if significant ($p<0.10$).

Significant interactions between agricultural status and the covariates were found on the Serial Digit Learning Test (AG × age; $F(1,122) = 3.96$, $p = 0.049$) and AG × gender; $F(1,122) = 4.28$, $p = 0.041$), the Symbol-Digit Test (AG × education; $F(1,127) = 4.20$, $p = 0.043$) and preferred hand Finger Tapping (AG × gender; $F(1,129) = 4.73$, $p = 0.031$). Table 4 contains scores for agricultural and non-agricultural controls adjusted to reflect the mean response for a 25 year old individual with 6 years of education; results are shown separately for each sex in cases where a significant AG × gender interaction was found. Details concerning the interactions involving agricultural status and either age or education can be found in Table 5. Scores on the Symbol Digit (latency) tests improve (i.e., decrease) significantly with increasing education for both groups, but those working in agriculture show greater benefit from each additional year of education. On the Serial Digit Learning test the two groups have linear trends that diverge with respect to age though neither trend is significant. The summary index, derived from 11 of the 16 NB tests, also exhibited an AG × gender interaction (F(1,129) = 6.51, *p* = 0.012).

[Table 4 and Table 5 should be added near here]

Discussion

To the best of our knowledge, this is the first study to report a correlation between occupation, pesticide residues in house dust, biological indicators of exposure and effects on neurobehavioral performance. While the sample is limited to a migrant farmworker population in Oregon, it is important because of its linkage of multiple points on the exposure and health effects pathway which forms the basis on studies of environmental and occupational exposure and health. Our studies of the Hood River agricultural community have documented higher levels of indoor pesticide contamination compared to our referent, agricultural and non-agricultural Oregon communities (McCauley et al. 2001). Farmworkers are exposed to pesticides from both work practices and from living in housing close to agricultural fields. Though not measured in this study, we have previously reported the proximity of farmworker housing to agricultural fields in this community to be on an average of 15 meters (McCauley et al. 2001).

Application dates in the spray records from orchards in the Hood River neighborhoods surrounding the homes of the participants in this study indicate that applications of phosmet and AZM in this region occurred within one week of our dust sample collection. The variability of levels of pesticide in household dust according to season and spraying activity has not been well established and would be difficult to ascertain in most agricultural communities because field specific information on . product name, amount applied at each location and the crop type are not available. At this time only five states had legislation requiring extensive reporting of pesticide use, including individual grower usage.

The correlation found between environmental contamination and levels of urinary metabolites is a further example of a take-home pathway of pesticide exposure and points to the importance of home hygiene practices to decrease take-home exposures (Coronado et al. 2004; McCauley et al. 2003). Overall the correlation found is impressive given that current pesticide levels is house dust is merely a marker of exposure history and not a direct measure. We have previously reported in a small sample of growers in Hood River a significant correlation between self-reported hygiene practices and levels of pesticides in home dust. It is important that health education messages to this community include information on measures that growers and farmworkers can do to prevent home contamination (Coronado et al. 2004; McCauley et al. 2003; Thompson et al. 2003). Of particular importance is the removal of work shoes outside of living areas, changing out of work clothes and showering upon arriving home, frequent mopping of hard floor surfaces, and steam cleaning carpets when appropriate. This was a community-based participatory research study and all of the study results have been shared with advisory board members and farmworkers in the community. We also have reported on the development and dissemination of a training video that emphasizes take-home pesticide contamination and the importance of home hygiene practices (Napolitano 2002).

Among individuals of similar age and education, we found that non-agricultural adults performed better on the majority of the neurobehavioral measures that we included in our testing protocol. Measuring neurobehavioral performance in immigrant, non-English speaking populations and obtaining comparable comparisons groups are always scientific challenges.

Participants from both groups studied in this report had been residing in the US for comparable periods of time and had similar years of education. Both groups have immigrated for similar areas of Mexico. Both groups tend to maintain strong ties with the recently immigrated families within their community. Most important the Latino community organizations within the state informed the researchers on the similarities of these two groups and how the tourism workers would be an appropriate comparison population to the farmworkers. Both groups are very similar in their engagement in low-paying jobs such as agricultural work, house-keeping or janitorial services for the hotel industry, or restaurant workers in a tourism community.

These findings add support to a growing body of evidence of neurobehavioral changes in occupational groups chronically exposed to pesticides (Bazylewicz-Walczak 1999; Fiedler et al. 1997; Kamel et al. 2003; Stephens et al. 1995). A pattern of poorer performance among farmworkers was observed on the majority of measures in our test battery. The performance measures that we found to be associated with agricultural work are also measures that have been shown to be associated with low-level, chronic exposures to pesticides including sustained attention, information processing and motor speed and coordination. (Simple Reaction Time, Symbol-Digit, Syntactic Reasoning, Pursuit Aiming).

In research conducted to date, the results of differences in performances on highly specific neurobehavioral tests have been the most common methodological approach. The correlation between the types of deficits seen, replication of specific deficits across studies, correlation with animal models, and the toxicological effects of these chemicals are no doubt of extreme importance. However Alavanja et al. (2004) and Heyer (1996) point out the utility of grouping results of neurobehavioral tests as a tool in interpreting findings because it will increase the power to detect effects of exposure in epidemiological investigations. We found the summary index useful in discerning differences in exposure groups and gender effects. We did however construct the summary index *a priori* to reflect components of all the major areas being tested. Selective Attention Latency and Continuous Performance Latency were not part of the summary index, but showed a significant correlation with the levels of urinary metabolites. Future methodological investigations of the utility of a neurobehavioral summary index are needed.

Interactions found between neurobehavioral performance and demographic variables such as age, education, and gender have been known to impact performance on neurobehavioral tests (Anger et al.1997). The neurobehavioral summary index score was significantly affected by the gender of the farmworker. The reasons for this difference are unclear. Previous studies of neurobehavioral performance in farmworkers have generally assumed that observed deficits are a result of pesticide exposure (Kamel 2003) and significant gender effects in humans have not been reported. Several findings examining OP exposure in rats have demonstrated differential effects of gender (Dam et al. 2000; Levin et al. 2001; Levin et al. 2002). In this study, male farmworkers tended to have higher levels of methyl metabolites than female workers. These differences could be contributed to cultural, exposure, metabolic or other yet unidentified factors. It is also important to consider genetic differences in the ability to metabolize organophosphate pesticides (Furlong 2000). Future studies will examine polymorphic differences and their relation with factors in the exposure pathway.

The design of this study has several limitations that deserve noting. Pesticide-specific information cannot be derived from quantitatively measuring the total urinary DAP metabolite levels and because individual pesticides differ in toxicity, these cumulative measurements can not be viewed as a measure of total toxicity (Wessels 2003). Furthermore, these biomarkers reflect recent exposure via all pathways over a very short time frame. Regulation of pesticides is changing, and the pesticides found in home dust will vary according to the types of crops grown in an area. Therefore, similar results may not be found in all agricultural communities. For example, in subsequent years after this study, AZM became less frequently used and the pattern of pesticides that we found in home dust in the same communities changed. If at all possible, future studies should include markers of specific organophosphate pesticides, rather than the DAPs. Reporting systems need improvement so that occupational spray records can be correlated with urinary levels of pesticides.

Urinary metabolites of organophosphate pesticides have a relatively short half life and it is unlikely that the performance on the neurobehavioral tests at a given test session is a temporary influence on performance measured at that given point in time. Rather the urinary metabolite levels should be considered a marker or approximation of a level of exposure, just as the neurobehavioral measures are a marker of performance that could change from one testing session to another. So while one could suggest that the differences observed between agricultural and nonagricultural communities is due to pesticide exposure, additional studies are merited.

Conclusions

The results of this study are important because of its linkage of multiple points on the pesticide exposure pathway which forms the basis of studies of environmental and occupational exposure and health. While there have been increasing reports in the literature of the extent of pesticide exposure in agricultural communities, few studies have included markers of potential health effects. The correlation between levels of pesticides in the home and pesticide urinary metabolites point to significant prevention and education implications, these messages are important to the farmworker community and to others in agricultural communities. To our knowledge, these data are the first report of a significant correlation between low levels of urinary pesticide metabolites and neurobehavioral function. The increasing number of reports of neurobehavioral performance deficits in workers with long-term exposure to pesticides is significant and points to the need for assurance that farmworkers received mandated pesticide safety training and that occupational bio-monitoring extend beyond those individuals who handle and apply pesticides. Finally, improved worker surveillance is needed to be able to estimate the extend of pesticide exposure among a workforce that moves frequently to meet the employment needs of multiple agricultural operations.

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Table 1. Organophosphate pesticides detected (µg/g) in farmworker housing in Hood River Oregon 1999 (*n* = 26).

	Pesticide						
	Diaz.	Meth. Para.	Chlor-pyrifos	Mal.	Phos.	AZM	Combined Total[a]
n detect (%)	20 (77)	16 (62)	24 (92)	21 (81)	25 (96)	18 (69)	
LOD	0.01	0.01	0.01	0.01	0.01	0.10	
Minimum	0.01	0.01	0.01	0.05	0.16	0.30	0.57
Mean (sd)	0.31 (0.23)	0.38 (0.60)	0.20 (0.24)	0.38 (0.40)	5.2 (4.1)	5.9 (4.5)	10 (6.5)
Median	0.31	0.06	0.13	0.18	4.4	5.3	9.4
Maximum	0.72	1.9	1.2	1.4	22	16	26

[a] sum of six organophosphate pesticide residues; non-detects replaced by ½ LOD prior to forming sum.

Table 2. Urinary metabolite levels (µmoles/L) in farmworkers at T1 and T2. Non-detects were replaced by one-half the LOD prior to computing summary statistics.

		%Detect[a]		Mean (sd)[b]		Median[c]	
		T1	T2	T1	T2	T1	T2
	LOD	(n=93)	(n=79)	(n=84)	(n=68)	(n=84)	(n=68)
DMTP	0.015	97	100	0.63 (0.79)	0.67 (0.67)	0.34	0.35
DMDTP	0.010	74	95	0.34 (0.69)	0.54 (0.88)	0.09	0.12
DETP	0.0095	34	33	0.04 (0.12)	0.02 (0.03)	0.00	0.00
DMTP + DMDTP	—	—	—	0.97 (1.40)	1.21 (1.46)	0.43	0.48
DMTP + DMDTP average T1 & T2‡				1.01 (1.08)		0.56	

[a] complete sample [b] valid urine samples only [c] at least one valid urine sample (n=88)

Table 3. Partial correlations between neurobehavioral performance and levels of combined thiomethyl metabolites adjusted for age, sex, and education.

Test	Partial correlation	1-sided p-value
Digit Span Forward*	0.122	0.861
Digit Span Backward*	0.144	0.871
Progressive Ratio*	–0.149†	0.088
Reaction Time*	0.155†	0.080
Selective Attention trials	–0.120†	0.139
Selective Attention ISI*	0.088†	0.214
Selective Attention Latency	0.251†	0.011
Serial Digit Learning*	0.063	0.711
Symbol Digit Latency*	0.281†	0.005
Finger Tapping (preferred)*	–0.252†	0.012
Finger Tapping (non-pref.)*	–0.132†	0.116
Finger Tapping (alternating)*	–0.208†	0.029
Continuous Performance		
% hits*	0.055	0.685
% correct rejects	0.043	0.647
hit latency	0.195†	0.042
false alarm latency	0.160†	0.092
Summary index	–0.184†	0.047

† higher levels of metabolites associated with poorer performance

* indicates test is a component of the summary index

Table 4. Mean score and standard error of the mean (SEM) for 16 neurobehavioral tests. Adjusted means correspond to a 25 year old individual with six years of education in their country of origin. The one-sided *p*-value tests whether performance within the agricultural population (AG) is lower than the non-agricultural control population (Non-AG).

	Mean (SEM)		
Test	AG	Non-AG	1-sided *p*-value
Digit Span Forward*	4.12 (0.17)	4.37 (0.19)	0.10†
Digit Span Backward*	3.86 (0.19)	4.53 (0.21)	< 0.01†
Progressive Ratio*	600.40 (14.53)	600.22 (16.44)	0.50
Reaction Time*	340.95 (10.50)	327.77 (11.89)	0.13†
Selective Attention Trials	450.27 (10.03)	456.16 (11.48)	0.31†
Selective Attention ISI*	397.85 (13.45)	386.19 (15.40)	0.23†
Selective Attention Latency	323.00 (6.64)	315.15 (7.60)	0.15†
Serial Digit Learning*			
Male	11.36 (1.31)	8.36 (1.57)	0.93
Female	9.33 (1.09)	11.56 (1.66)	0.13†
Symbol-Digit*	3034.58 (113.74)	2973.38 (158.38)	0.38†
Finger Tapping Preferred*			
Male	99.80 (2.69)	96.88 (3.39)	0.75
Female	81.68 (2.31)	90.41 (3.60)	0.02†
Finger Tapping Non-pref.*	89.22 (2.51)	90.75 (2.84)	0.30†
Finger Tapping Altern.*	52.25 (3.00)	46.72 (3.42)	0.95
Continuous Performance			
Percent hits*	0.90 (0.02)	0.88 (0.02)	0.84
Percent correct rejects	0.95 (0.01)	0.97 (0.01)	0.26†
Hit latency	407.82 (10.38)	396.55 (11.63)	0.17†
False alarm latency	483.36 (21.40)	494.16 (24.88)	0.67
Summary index			
Male	1.01 (0.32)	0.18 (0.38)	0.95
Female	–1.00 (0.25)	–0.04 (0.39)	0.02†

* indicates test is a component of summary index

† indicates Non-AG performed better than AG

Table 5. Beta coefficients from significant interactions in a regression model used to compare neurobehavioral performance between AG and non-AG subjects. For each neurobehavioral test below, the coefficient shows the change in average performance for each additional year of age or education.

Test (interaction)	beta (se)	*p*-value
Serial Digit Learning (AG × age)		
Non-AG	0.26 (0.17)	0.13
AG	-0.16 (0.12)	0.20
Symbol Digit Latency (AG × education)		
Non-AG	-90.87 (40.17)	0.03
AG	-197.52 (33.12)	<0.01

Figure 1. Scatterplot of combined methyl residues found in dust versus thiomethyl metabolite concentration in urine (*n*=23 pairs). Spearman's correlation is 0.47 (1-sided *p* = 0.013).