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Neurological outcomes among pesticide applicators

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NEUROLOGICAL OUTCOMES AMONG PESTICIDE APPLICATORS

by

Sarah Elizabeth Starks

An Abstract

Of a thesis submitted in partial fulfillment
of the requirements for the Doctor of
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Thesis Supervisor: Professor Fredric Gerr

ABSTRACT

The acute nervous system toxicity of organophosphate (OP) pesticides is well described. However, the reported long-term effects of OP pesticides on the nervous system are inconsistent. This inconsistency may be due to imprecise estimates of pesticide exposure, variability of central nervous system (CNS) and peripheral nervous system (PNS) assessment, small samples, and poor control of confounding.

The primary goal of this research was to examine the association between long-term OP pesticide use on CNS and PNS function among pesticide applicators. An additional goal was to examine the association between high pesticide exposure events (HPEEs), which typically do not result in acute toxicity, and CNS function. Study participants were recruited from among applicators enrolled in the Agricultural Health Study (AHS) in Iowa and North Carolina. In 2006-2008, 701 male pesticide applicators completed a battery of neurobehavioral (NB) and neurological tests. Information about individual pesticide use was obtained from previous AHS interviews and a questionnaire administered during NB testing. Associations between pesticide use and neurological outcomes were estimated with linear and logistic regression models while controlling for covariates.

When associations were examined between agent-specific pesticide use and nine NB tests, significantly poorer performance was observed on four tests and significantly better performance on five tests. Additionally, for some pesticides, we observed differential associations by state, suggesting that regional differences in pesticide practices may influence neurotoxicity. Overall, our results did not provide strong evidence that OP pesticide use was associated with adverse NB test performance.

A history of at least one HPEE was reported by 23 percent of participants. Significant adverse associations were observed between HPEEs and two of the nine NB tests. Participants with HPEEs were, on average, 4.9 seconds slower on a test of visual

scanning/processing, and 2.2 seconds slower on a test of visual scanning/motor speed. Overall, small but meaningful associations were observed between HPEEs and adverse CNS function.

When associations were examined between pesticide use and PNS function, five of six neurological physical examination outcomes were associated with ever-use of one or more OP pesticides. Odds ratios ranged from 1.9 to 3.1. However, mostly null associations were observed between OP pesticide use and electrophysiological tests, hand strength, sway speed and vibrotactile threshold. This study provides some evidence that long-term exposure to OP pesticides is associated with impaired PNS function.

In summary, our results suggest that exposure to a few individual OP pesticides as well as HPEEs may contribute to adverse neurological function. The observed exposure-effect associations were present after adjustment for confounding and were independent of past-diagnosed pesticide poisoning. We believe this research contributes important new evidence to an inconsistent literature. Reducing pesticide exposure and preventing HPEEs among pesticide applicators remain important public health goals.

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Graduate College
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PH.D. THESIS

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To Mom

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In summary, our results suggest that exposure to a few individual OP pesticides as well as HPEEs may contribute to adverse neurological function. The observed exposure-effect associations were present after adjustment for confounding and were independent of past diagnosed pesticide poisoning. We believe this research contributes important new evidence to an inconsistent literature. Reducing pesticide exposure and preventing HPEEs among pesticide applicators remain important public health goals.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Pesticides are used throughout the world to protect crops from damage caused by insects, weeds, fungi, rodents and other harmful pests. Over the years, pesticides have vastly improved crop quality and yield and enhanced human health by controlling insect-borne diseases. However, there are negative consequences of pesticide use. Many pesticides are poorly selective and are toxic to non-target species, including humans. These chemicals pose substantial occupational health risks, especially to those with the greatest risk of exposure, such as agricultural workers.

Neurotoxicity, defined as the ability of a chemical to produce an adverse effect on the nervous system, is perhaps the best-documented health effect of pesticide exposure in humans. Although many pesticides have some level of neurotoxicity, insecticides are the most acutely neurotoxic to humans and other non-target species compared to other pesticides [1]. Most insecticide pesticides, including the carbamates, organochlorines, organophosphates, pyrethrins and synthetic pyrethroids, kill insects by disrupting their nervous system. These chemicals have varying levels of toxicity; however, the adverse health effects of organophosphate pesticides, which are more commonly used, are of particular concern in agricultural populations and are the primary focus of this research.

Organophosphate Pesticides

Organophosphate (OP) pesticides constitute the most widely used subclass of insecticides and are a leading cause of pesticide-related morbidity and mortality throughout the world. Although the amount of OP pesticide use in the United States has declined by 45 percent since the 1980's, an estimated 73 million pounds of OP pesticides

were used in 2001 [2]. Of these, approximately 20-25 million pounds of malathion and 8-10 million pounds of chlorpyrifos were used in the agricultural sector alone [2].

Exposure to OP pesticides can occur through gastrointestinal, respiratory and dermal routes. High oral doses are usually the result of accidental or intentional ingestion and often lead to severe poisoning or death. According to the World Health Organization (WHO), there are an estimated three million severe pesticide poisoning cases each year that result in more than 200,000 fatalities, worldwide [3]. Most of these cases involve OP agents. Low oral doses of pesticides are ingested by the general population as contaminates in drinking water or as pesticide residues in food [1]. Among agricultural workers, the dermal route offers the greatest potential for exposure with some contribution of inhalation when aerosols are used. Workers involved with mixing, loading, transporting and application of pesticides are at the greatest risk for pesticide exposure [1].

The acute toxicity of a pesticide refers to its ability to cause systemic effects within minutes to hours of a single exposure. The acute toxicity of OP pesticides on the nervous system and other organs is well described and results from the inhibition of the enzyme acetylcholinesterase (AChE). Sufficient inhibition of AChE leads to the accumulation of the neurotransmitter acetylcholine, resulting in excessive cholinergic activity in the nervous system [4]. Mild cases of acute poisoning display symptoms such as headache, dizziness, salivation, sweating, nausea, vomiting and diarrhea. More severe cases may develop cardiac rhythm disturbances, muscular fasciculation, convulsions or coma; death generally occurs as a result of respiratory failure.

An “intermediate syndrome” has also been described as a late complication of some cases of severe acute OP exposure, and is characterized by proximal muscle weakness lasting 5-18 days [5-6]. Exposure to OP pesticides that inhibit the enzyme *neuropathy target esterase* results in a delayed-onset peripheral neuropathy, (*organophosphate induced delayed neuropathy* or OPIDN) which occurs weeks to

months following acute exposure. Clinical manifestations of OPIDN include sensory loss, muscle weakness and flaccidity of the distal skeletal muscles of the lower and upper extremities and ataxia [7-8]. Incomplete recovery follows removal from exposure.

Long-term, repeated exposures to low or moderate-levels of OP pesticides generally do not inhibit AChE sufficiently to cause overt signs of cholinergic toxicity [7]. There is some evidence, however, that long-term low-level exposure to OP pesticides may produce adverse neurological effects, including neurobehavioral changes and impaired peripheral nerve function. Additionally, long-term OP exposure may increase the risk of some neurodegenerative diseases such as Parkinson's disease [9] and Alzheimer's disease [10]. Epidemiological studies of long-term OP pesticide exposure are presented on page 4.

Neurobehavioral and Neurological Testing among Organophosphate Exposed Humans

The literature is not definitive as to which neurological functions may be affected by OP pesticide exposure. Therefore, a battery of tests is often used to assess a wide range of neurological domains among pesticide exposed individuals. Peripheral nervous system function can be assessed with numerous tests including neurological physical examination tests, electrophysiological studies (nerve conduction studies), vibrotactile threshold testing, hand strength dynamometry and standing steadiness (also known as postural stability or sway). Central nervous system function is generally assessed with a set of neurobehavioral and neuropsychological tests. In 1983, the first testing battery for investigating occupational neurotoxicity was developed. This battery, the "Neurobehavioral Core Test Battery" (NCTB), was developed by the World Health Organization (WHO) and the U.S. National Institute for Occupational Safety and Health (NIOSH) and included seven tests believed to be sensitive to neurotoxic chemicals [11].

The NCTB included the Digit-Symbol Substitution test, Digit Span, Benton Visual Memory Test, Pursuit Aiming II, Simple Reaction time (SRT), Santa Ana Dexterity Test, and Profile of Mood States questionnaire (POMS). The first computer-based testing system, called the "Neurobehavioral Evaluation System (NES)" was developed by Baker and Letz in 1985 and included 22 behavioral tests used to evaluate central nervous system function [12]. A major advantage of the computer-based NES testing battery over the manually-administered NCTB was that testing conditions were easily reproducible, administration of the tests was simple and required little training and testing results were immediately available. Two additional versions of the NES testing battery were later released (NES2 and NES3) [13]. The Neurobehavioral Evaluation System was the most widely used computer-based testing system in the 1990s and has been used more extensively than any other testing battery in behavioral neurotoxicity research [11].

There is currently no consensus as to which individual neurobehavioral tests should be used in neurotoxicity assessments. Extensive cross-sectional research has identified numerous tests that detect effects of neurotoxic substances. Tests that have most frequently revealed group differences include: Digit-Symbol; Digit Span; Continuous Performance Test; Simple Reaction Time (SRT); and Finger Tapping [11, 14-15].

Epidemiological Studies of Long-term Organophosphate Exposure

Numerous studies have reported long-term neurological and neurobehavioral sequelae following a pesticide poisoning event [16-21]. However, the consequences of long-term exposure at levels insufficient to cause clinical toxicity are more controversial. Previous investigations have shown mostly inconsistent results. The inconsistency in observed associations is potentially due to a number of methodological limitations,

including imprecise estimates of pesticide exposure, variability in assessment of central and peripheral nervous system function, small samples with fewer than 50 exposed individuals, and poor control of confounding. The literature examining the neurological effects of long-term OP pesticide exposure, without previous known pesticide poisoning, is briefly reviewed below to better illustrate the inconsistencies in the current state of knowledge. The review is limited to occupational studies of adult populations published in the past 20 years. A summary of studies examining the association between long-term OP use and central and peripheral nervous system function is presented in Tables 1.1-1.2.

Studies of Central Nervous System Function

Ames et al (1995) studied 45 male subjects with documented cholinesterase inhibition (with no evidence of frank pesticide poisoning) and 90 male subjects with no past cholinesterase inhibition or current pesticide exposure [22]. Eight neurobehavioral tests from the Neurobehavioral Evaluation System (NES) were administered to study participants (Mood Scales, Finger Tapping, Sustained Attention, Hand-eye Coordination, Simple Reaction Time, Digit-Symbol, Pattern Memory, and Serial Digit Learning). After adjustment for covariates, only one of the eight tests (Serial Digit) was significantly associated with exposure status. However, this outcome was associated with improved neurobehavioral performance.

Stephens et al (1995) administered neurobehavioral tests to 146 sheep farmers exposed to OP pesticides and 143 unexposed quarry workers [23]. A battery of eight neurobehavioral tests were administered to study subjects (Simple Reaction Time, Digit-Symbol, Digit Span, Syntactic Reasoning, Category Search Classification and Recognition, Visual Spatial Memory and Serial Word Learning). After adjusting for confounding, sheep farmers performed significantly poorer than referents on the Digit-Symbol and Syntactic Reasoning tests.

Fiedler et al (1997) evaluated neurobehavioral outcomes among 57 OP pesticide exposed male fruit tree farmers and 42 age-matched OP pesticide exposed cranberry growers and hardware store owners with no previous OP exposure [24]. Neurobehavioral tests of concentration, visuomotor skills, memory, expressive language and mood were administered. The unexposed participants were significantly better educated than the exposed group. No pesticide specific information was presented. After controlling for confounders, slower Simple Reaction Time was significantly associated with exposure group status and a metric of lifetime exposure. The authors concluded that "demonstrable neurobehavioral performance deficits in an asymptomatic population are, at most, subtle".

Bazylewicz-Walczak et al (1999) administered the Neurobehavioral Core Test Battery (NCTB) to 26 female greenhouse workers with regular OP pesticide exposure and 25 unexposed referents matched for age, sex, education, and place of residence [25]. Testing was performed before the spraying season and again after the spraying season. The most frequently used OP pesticides were dichlorvos, methamidophos, methidathion, and primiphos-methyl. Exposure dosimetry (based on dermal and respiratory measurements) suggested low OP exposure levels. Statistically significant exposure group effects were observed for tests of Simple Reaction Time and hand-eye coordination (Aiming). Participation rates were not provided.

Steenland et al (2000) studied neurological function among 191 current and former chlorpyrifos-exposed termite exterminators and 198 unexposed comparison subjects [26]. The comparison population consisted of two unexposed groups (100 friend controls and 98 state workers). Tests of both central and peripheral nervous system function were administered, including the Neurobehavioral Evaluation System (NES), vibrotactile threshold, arm/hand tremor, postural sway, manual dexterity, eye-hand coordination, visual acuity and color vision, olfaction, nerve conduction velocity, neurological clinical examination and neurological symptoms questionnaire. The average

duration of exposure was 2.4 years for chlorpyrifos and 2.5 years for other pesticides. The exposed group did not differ significantly from the unexposed group for any clinical examination outcome. Longer sway paths were observed among the exposed than the unexposed. No significant differences were observed between the exposed and unexposed groups on most of the neurobehavioral tests. Exposed participants reported significantly more "fatigue" and "tension". The authors reported that 90% of the applicators in the study were under 50 years of age and that, if chlorpyrifos exposure causes delayed neurotoxic effects that become apparent with age, these effects would have been missed. Furthermore, applicators with a relatively short duration of exposure (2 years) to chlorpyrifos were studied, possibly limiting the ability to observe exposure effects.

Farahat et al (2003) studied 52 male workers occupationally exposed to OP pesticides and 50 unexposed male controls who were similar in age, socioeconomic class and years of education [27]. The study was conducted during the period when pesticides were applied to cotton crops. Physical examinations and neurobehavioral tests were performed on all subjects. The unexposed group had slightly lower educational level than the exposed group. After adjustment for confounders, significant associations with exposure category were observed for several neurobehavioral tests including Similarities, Digit-Symbol, Trails A&B, Letter Cancellation, Digit-span forward and backward, and Benton Visual Retention. Elevated odds ratios were observed for numerous symptoms. The authors concluded that exposure to OP pesticides "is associated with deficits in a wider array of neurobehavioral function than previously reported". Participation rates were not provided.

Kamel et al (2005) conducted a cross-sectional analysis of 18,782 male licensed pesticide applicators enrolled in the Agricultural Health Study (AHS) [28]. Pesticide applicators provided information on lifetime OP use and 23 neurological symptoms. After adjusting for covariates, cumulative lifetime days of OP pesticide use was

significantly associated with greater symptom count category (defined as reporting ≥ 10 neurological symptoms). Furthermore, lifetime OP pesticide use was significantly associated with 22 of the 23 individual neurological symptoms with odds ratios ranging from 1.26 to 2.67. Significant adverse associations were independent of high pesticide exposure events and recent pesticide use (within the past 12 months).

Roldan-Tapia et al (2005) conducted a cross-sectional study of 40 male greenhouse spraying workers with long-term exposure to OP pesticides and 26 unexposed controls [29]. Twenty-one neurobehavioral tests were administered to the study subjects. After controlling for covariates, workers with greater than 10 years of exposure to OP pesticides performed significantly poorer on tests of visuomotor praxis (Rey-Osterreich figure copy quality), integrative task performance time (Rey-Osterreich figure copy time) and perceptive function performance (Benton visual form description). Limitations of the study include the small size of the sample and the fact that examiners were not blinded to the subjects' exposure status.

Rothlein et al (2006) performed neurobehavioral testing on 92 migrant agricultural workers and 45 non-agricultural workers [30]. Sixteen neurobehavioral tests were selected and included measures of psychomotor and cognitive functioning. Multiple linear regression methods were used to compare neurobehavioral tests performance between the agricultural and non-agricultural groups while controlling for age, years of education and sex. The authors report that the non-agricultural controls performed better on the neurobehavioral tests. However, with the exception of Digit-Span, differences between the groups were not statistically significant. No information was collected on specific pesticide use or the duration of time working with pesticides.

Studies of Peripheral Nervous System Function

Stokes et al (1995) conducted a study of 68 male pesticide applicators and 68 population based referents, matched on age, sex and county of residence, to examine the association between OP pesticide exposure and peripheral nervous system function [31] . Vibration threshold sensitivity was used as an indicator of chronic neurotoxicity and was measured for both the upper and lower extremities. Pesticide applicators had sprayed OP pesticides for an average of 20 years. Pesticide application was associated with a significant increase in dominant and non-dominant hand vibration threshold (i.e. poorer sensory performance) among applicators compared to referents. A non-significant elevation was observed for the dominant and non-dominant foot. The authors did not control for height, which is a known predictor for vibration threshold in the upper and lower extremities [32] . Furthermore, information about previous pesticide poisoning events was not reported.

Cole et al (1998) conducted a cross-sectional study to evaluate peripheral nervous system function among three groups with differing levels of OP exposure and an unexposed referent group [33]. Participants were 123 exposed pesticide applicators, 28 exposed non-applicators, 23 female farm members with little pesticide exposure and 72 unexposed local town residents. After adjusting for confounders, significant differences were observed for peripheral nerve symptoms, abnormal deep tendon reflexes, signs of poor coordination signs, and reduced muscle power when the most heavily exposed of the three exposure groups was compared to the referents. Mean toe vibration threshold scores were significantly higher in pesticide applicators compared to controls. Exposed applicators had used OP pesticides a mean of 111 hours during the month prior to testing, therefore the results of this study may reflect a mixing of acute and chronic effects.

Engel et al (1998) conducted a cross-sectional study of 67 Hispanic farm workers (apple thinners) and 68 referent subjects matched on age, gender, ethnicity, and education

[34]. Nerve conductivity and measures of neuromuscular junction function were performed on all subjects. Farm workers with 80 or more hours of work as an apple thinner during the current growing season were eligible to participate. Individuals were excluded if they reported mixing, loading or applying pesticides during the six months prior to enrollment. The pesticides used in the apple orchards were primarily azinphosmethyl and "possibly" phosmet or methyl parathion. No statistically significant neurophysiological differences between the exposed and references groups were observed.

London et al (1998) conducted a cross-sectional study of 164 pesticide applicators and 83 non-spraying reference workers on deciduous fruit farms [35]. Neurological symptoms, vibration perception, and tremor were assessed during the spraying season. Exposure was derived with the use of a job exposure matrix for pesticides in agriculture. Specific chemicals used were not reported. Strong associations were observed between neurological symptoms and exposure status. However, no significant associations were observed between lifetime OP pesticide use and any of the outcome measures. The prevalence of alcohol abuse, medical illness, and previous head injury were high and may have inflated the outcome measure variance.

Pilkington et al (2001) administered a neurological symptoms questionnaire and measured sensory thresholds (hot sensation, cold sensation and vibration) among 612 OP-exposed sheep dipping farmers and 160 unexposed comparison subjects [36]. The comparison group consisted of 53 farmers with no sheep dipping experience and 107 ceramic workers. Sheep dippers were on average six years older than the other groups and included a higher proportion of women (14% vs. 6%). After adjusting for confounding, significant associations were observed between cumulative exposure to OP pesticides and the frequency of neurological symptoms. There was no evidence of an association between cumulative OP exposure and tests of sensory thresholds. No information about past poisoning episodes was provided.

Peiris-John et al (2002) performed neurological physical examinations and nerve conduction tests on 30 farmers who regularly sprayed OP pesticides and 30 unexposed fisherman [37]. The evaluations of study subjects were performed once between cultivation seasons and once during the cultivation season. The mean duration of exposure to pesticides among the farmers was 14 years before the current cultivation season; all farmers had reported using pesticides within the preceding month. Between cultivation seasons, significant differences between farmers and controls were found for sensory conduction velocity and motor conduction velocity. However, sensory conduction velocity among farmers was significantly greater, whereas the motor conduction velocity was significantly lower compared to the referent group. During the cultivation seasons there were no differences in sensory or motor conduction velocities between the two groups. No information was provided on previous pesticide poisoning events.

Albers et al (2007) conducted a prospective cohort study and evaluated peripheral nervous system function among 113 chemical workers at the Dow Chemical Company [38]. Nerve conduction tests were performed on 53 chlorpyrifos manufacturing workers and 60 referent workers. Industrial hygiene records were used to establish estimates of chlorpyrifos exposure from the time of initial employment to the baseline examination (historic chlorpyrifos exposure). Median motor forearm conduction velocity, median motor F-wave latency and a summary Z score for sensory conduction were significantly associated with historic chlorpyrifos exposure. Three additional outcomes (median sensory amplitude, ulnar sensory terminal conduction velocity, and a summary Z score for motor conduction) showed borderline-significant associations (p-value range 0.06-0.08). Few dose-effect relationships were observed among participants with historic chlorpyrifos exposure that exceeded 20 mg/m^3 days.

El-Helaly et al (2009) examined peripheral nerve function among workers with long-term exposure to OP pesticides [39]. Participants included 36 male OP pesticide

sprayers and 26 male unexposed sanitation workers. A neurological physical examination and nerve conduction testing (23 tests of motor nerve conduction and 18 tests of sensory nerve conduction) were performed on all subjects. The mean duration of pesticide exposure among the OP sprayers was 9.4 years. Malathion (100%), dimethoate (97%) and fenthion (83%) were the most commonly used OP pesticides. Neurological signs and symptoms were not significantly different between OP sprayers and the comparison group. However, the OP sprayers had significantly worse results on 16/23 motor nerve conduction studies and 10/18 sensory nerve conduction studies in comparison to the referent group. Furthermore, the prevalence of diagnosed peripheral neuropathy was significantly higher among OP sprayers (34%) than among the controls (5%). Due to the small sample size, investigators were unable to assess exposure to individual pesticides.

Critique of the Literature

Seventeen studies examining the association between long-term OP pesticide exposure and neurological outcomes were reviewed. These studies were limited to adult working populations with no previous history of pesticide poisoning. While most of these studies provided some evidence of an association between long-term OP pesticide exposure and adverse CNS or PNS function, the results were far from consistent. A number of methodological weaknesses may have contributed to the observed heterogeneity of effects. These weaknesses include imprecise pesticide exposure assessment, variability in neurological outcome measures, small sample sizes and inadequate control of confounding.

Imprecise or inaccurate estimation of pesticide exposure is a frequent criticism of prior studies. Cumulative pesticide exposure was assessed in five studies [28-29, 35-36, 38]. However, most of the studies used a dichotomized exposure metric (e.g. agricultural workers vs. non-agricultural workers) [22-27, 30-31, 33-34, 37, 39]. Exposure estimation

using dichotomized exposure metrics such as job title or occupational classification results in the mixing of a wide range of actual exposures into a few categories. Consequently, exposure information is lost and observed associations are likely to underestimate true associations.

Measures of neurological function varied widely among the studies reviewed. Of the nine studies examining central nervous system function, one study assessed only neurological symptoms [28]. The remaining eight studies used computerized neurobehavioral tests which measured a variety of functional areas (i.e. cognitive, sensory-motor, psychological and psychomotor). Several studies reported using standard neurobehavioral tests such as the WHO Neurobehavioral Core Test Battery (NCTB) [25, 29], the Neurobehavioral Evaluation System (NES) [22, 24, 26] and the Behavioral Assessment and Research System (BARS) [30].

Among the eight studies examining associations between long-term pesticide exposure and peripheral nervous system function, one study examined only vibration thresholds [31], three studies examined vibration thresholds and neurological signs and symptoms [33, 35-36], three studies assessed only nerve conduction outcomes [34, 37, 40] and one study examined both neurological signs and symptoms and nerve conduction outcomes [39].

Another important limitation of the existing literature is small sample sizes. Of the 17 studies reviewed, five studies had fewer than 50 exposed individuals [22, 25, 29, 37, 39] and 11 studies had fewer than 100 exposed individuals [22, 24-25, 27, 29-31, 34, 37-39]. Studies with small sample sizes have low statistical power and produce imprecise estimates of exposure-response relationships. Furthermore, participation rates were not reported in some studies [22, 25, 27, 33]. Low participation rates may have resulted in the study of a non-representative sample.

Finally, a few of the reviewed studies had inadequate control for confounding factors. Age, education, and premorbid intellectual ability are known to influence

performance on neurobehavioral tests [41]. Although all of the studies examining neurobehavioral performance controlled for the effects of age and education, only one study examined premorbid intellectual ability [22]. Height is an important covariate of nerve conduction and vibrotactile threshold [42-43]. However, two studies using these measures of peripheral nerve function failed to control for the effects of height [31, 36]. Agricultural workers who use OP pesticides are often exposed to numerous other neurotoxic agents, including other classes of pesticides (e.g. carbamates), organic solvents, and metal fume generated by welding. Few studies characterized exposure to other neurotoxicants. Consequently, failure to adequately control for these confounding factors could lead to inaccurate conclusions regarding the exposure-effect association. In addition, several studies used referent groups that may have differed from the exposure group on important characteristics other than pesticide exposure. Furthermore, referent groups selected from the same community or place of employment as the exposed group may not truly represent an unexposed referent group. Use of referent participants with a non-zero level of exposure will likely lead to observed associations smaller than those that would have been observed had the referent participants been completely free of exposure.

In sum, methodological limitations are common in the existing literature. Results of well-designed studies using more accurate estimations of pesticide exposure, standard measures of neurological function and larger sample sizes, and better control of potential confounding factors may be more consistent than the current literature.

Specific Aims

Despite numerous studies, associations observed between pesticide exposure and neurological function are inconsistent. The primary goal of this research was to better estimate the association between long-term, low-level, pesticide exposure and central and

peripheral nervous system function. Three studies were conducted to achieve this goal. The Specific Aims of the three studies were:

Specific Aim 1: To model exposure-effect associations between estimates of cumulative lifetime exposure to OP pesticides and neurobehavioral measures of central nervous system (CNS) function.

Specific Aim 2: To model exposure-effect associations between high pesticide exposure events (HPEEs) and neurobehavioral measures of central nervous system (CNS) function.

Specific Aim 3: To model exposure-effect associations between estimates of cumulative lifetime exposure to OP pesticides and neurological measures of peripheral nervous system (PNS) function.

Significance of this Research

According to the U.S. Bureau of Labor Statistics, over 800,000 persons held jobs as agricultural workers in 2008 [44]. Given the large number of workers who are potentially exposed to OP pesticides, adverse neurological and neurobehavioral health effects are likely to have public health significance. The research presented in this dissertation was conducted to examine associations between pesticide exposures, at levels insufficient to cause clinical toxicity, and neurological and neurobehavioral function among pesticides applicators.

Table 1.1. Summary of studies examining the association between long-term OP pesticide exposure and measures of central nervous system (CNS) function in non-poisoned populations (n=9)

Author and year	Study population	OP exposure	CNS outcome measures	Main findings	Comments
Ames, 1995 [22]	45 male agricultural workers, 90 unexposed males	Agricultural workers with documented cholinesterase inhibition	8 NB tests	Significantly better performance on Serial Digit	Participation rates were not reported; exposed participants were10 years older than referents; unable to examine individual pesticides
Stephens, 1995 [23]	146 sheep farmers, 143 quarry workers	OP pesticide exposed sheep dip farmers	8 NB tests	Significant adverse performance on Digit-Symbol and Syntactic Reasoning	A significant dose-group association was observed for Syntactic Reasoning but not for Digit-Symbol; did not report individual pesticide use
Fiedler, 1997 [24]	57 male fruit tree farmers, 22 unexposed male blueberry/ cranberry growers, 20 unexposed hardware store owners	Fruit tree farmers with OP pesticide exposure	18 NB tests	Significantly slower performance on Simple Reaction Time (both dominant and non-dominant hands)	39% of eligible exposed, 14% of blueberry/cranberry farmers and 8% of hardware store owners participated; unexposed subjects were significantly better educated than the unexposed groups
Bazylewicz-Walczak, 1999 [25]	26 female greenhouse workers, 25 unexposed referents	Greenhouse workers with OP pesticide exposure	6 NB tests	Significantly slower performance on Simple Reaction Time and Aiming	Exposure level of greenhouse workers was low; participation rates were not provided
Steenland, 2000 [26]	191 current and former termiticide applicators, 198 unexposed comparison subjects (100 friend controls and 98 state workers)	Termiticide applicators exposed to chlorpyrifos	6 NB tests; 5 Mood scale tests; 2 Pegboard tests; 24 Symptom tests	Exposed subjects performed worse on the pegboard test (p=0.07) and reported significantly more symptoms of fatigue and tension	The average duration of chlorpyrifos exposure was 2.4 years
Farahat, 2003 [27]	52 male pesticide applicators, 50 unexposed male clerks	Pesticide applicators with OP pesticide exposure	12 NB tests; 11 Symptom tests	Exposed subjects performed significantly poorer on Similarities, Digit-symbol, Trailmaking A&B, Letter Cancellation, Digit Span and Benton Visual Retention; symptoms of dizziness and fatigue were significantly higher in the exposed group	The study was conducted at the time pesticides were applied to crops; participation rates were not provided

Table 1.1. Continued

Author and year	Study population	OP exposure	CNS outcome measures	Main findings	Comments
Kamel, 2005 [28]	18,782 male licensed pesticide applicators	Cumulative lifetime days of OP pesticide use	23 neurological symptoms	Greater symptom count was significantly associated with cumulative lifetime days of OP pesticide use; 22/23 symptoms were significantly associated with OP pesticide use	Significant adverse associations were independent of high pesticide exposure events and recent exposure
Roldan-Tapia, 2005 [29]	40 male greenhouse spraying workers, 26 unexposed controls	Years of OP exposure	21 NB tests	Significant adverse performance on 3 tests (Rey-Osterreich figure copy quality and figure copy time and Benton visual form discrimination) among workers with >10 years of exposure	Examiners were not blinded to the subjects' exposure status; dose-effect was not examined because of small numbers
Rothlein, 2006 [30]	92 agricultural workers, 45 non-agricultural workers	Agricultural workers with OP pesticide exposure	16 NB tests	Agricultural workers performed worse on 12 NB tests but only the Digit-span test was statistically significant	Duration of time working with pesticides was not reported; unable to examine individual pesticides

Table 1.2. Summary of studies examining the association between long-term OP pesticide exposure and measures of peripheral nervous system (PNS) function in non-poisoned populations (n=8)

Author and year	Study population	OP exposure	PNS outcome measures	Main findings	Comments
Stokes, 1995 [31]	68 pesticide applicators, 68 population based controls	Pesticide applicators with OP pesticide exposure	Vibration threshold sensitivity (upper/ lower extremities)	Significantly adverse vibration threshold for dominant and non-dominant hands among pesticide applicators	Vibration thresholds for dominant and non-dominant feet were greater in applicators compared to controls, but not significant; height was not controlled for in the analysis
Cole, 1998 [33]	123 pesticide applicators, 28 exposed non-applicators, 23 female farm members with little exposure, 72 unexposed controls	Farm workers (applicators and non-applicators) exposed to OP pesticides	5 neurological signs and symptoms; lower extremity vibration threshold	Applicators had significantly greater odds for peripheral nerve symptoms, signs of poor coordination, abnormal deep tendon reflexes, reduced muscle power and vibration threshold	Exposed participants had both current and long-term OP pesticide exposure; participation rates were not reported
Engel, 1988 [34]	67 apple thinners, 68 matched controls	OP-exposed workers who had worked at least 80 hours during the current season	Sensory and motor nerve conduction tests; neuromuscular junction testing	No significant differences were observed	Participation rates were high (99%); workers who had mixed loaded or applied pesticide in the past 6 months were excluded
London, 1998 [35]	164 male pesticide applicators, 83 male non-spraying reference workers	Average intensity of lifetime OP pesticide exposure	12 neurological symptoms; tests of vibration sense and tremor	Applicators reported significantly more dizziness, sleepiness and headache compared to referents; No significant associations between lifetime OP exposure and PNS outcomes	47% of the applicators reported applying OP pesticides in the preceding 10 days; 22% applied OP pesticides in the morning before their examination; high prevalence of head injuries and alcohol abuse
Pilkington, 2001 [36]	612 sheep dipping farmers, 53 farmers with no sheep dipping experience, 107 unexposed ceramic workers	Total number of days sheep dipping and cumulative exposure to OP dips	Neurological symptoms; thermal and vibration sensory testing	There was a weak adverse association between cumulative OP exposure and neurological symptoms; no evidence of an association between cumulative OP exposure and vibration or sensory measures	Only individuals who worked on a farm within the previous 12 months were invited to participate; No information about past pesticide poisoning events was provided

Table 1.2. Continued

Author and year	Study population	OP exposure	PNS outcome measures	Main findings	Comments
Peiris-John, 2002 [37]	30 farmers who sprayed OP pesticides, 30 unexposed fisherman	OP-exposed workers	5 nerve conduction studies (sensory conduction velocity, sensory latency, motor conduction velocity, motor amplitude and motor latency)	Sensory conduction velocity was significantly higher in farmers; motor conduction velocity was significantly lower in farmers	No significant difference in cholinesterase activity between farmers and controls; No information about past pesticide poisoning was provided
Albers, 2007 [38]	53 chlorpyrifos manufacturing workers; 60 referent workers	Historic and interim cumulative chlorpyrifos exposure	16 nerve conduction tests on the median, ulnar, and sural sensory nerves, and the median and peroneal motor nerves; 4 summary Z-scores	3 tests showed significant associations with historic chlorpyrifos exposure; 3 tests showed borderline-significant associations with historic chlorpyrifos exposure	Few dose-effect relationships were observed among participants with historic chlorpyrifos; participants were well-educated and employed in a carefully controlled work environment
El-Helaly, 2009 [39]	36 male OP sprayers, 26 unexposed sanitation workers	OP-exposed workers	Neurological signs and symptoms; 23 motor nerve conduction studies; 18 sensory nerve conduction studies	No significant differences on neurological signs and symptoms between workers and controls; OP sprayers had significantly worse results on 16/23 motor nerve conduction studies and 10/18 sensory nerve conduction studies compared to controls	Authors did not report if neurological testing was conducted during the spraying season; unable to examine individual OP pesticides

CHAPTER II

NEUROBEHAVIORAL OUTCOMES AMONG PESTICIDE APPLICATORS

Abstract

While acute organophosphate (OP) pesticide exposure is associated with adverse central nervous system (CNS) outcomes, little is known about the neurotoxicity of long-term exposure to OP pesticides that do not result in acute poisoning. To examine associations between long-term pesticide use and adverse CNS outcomes, neurobehavioral (NB) tests were administered to licensed pesticide applicators enrolled in the Agricultural Health Study (AHS) in Iowa and North Carolina. In 2006-2008, 666 male participants completed nine NB tests to assess memory, motor speed and coordination, sustained attention, verbal learning and visual scanning and processing. Ever-use and lifetime days of use of 16 OP pesticides were obtained from AHS interviews in 1993-2006. The mean age of participants was 61 years (SD = 12). Associations between pesticide use and NB outcomes were estimated with linear regression controlling for age and outcome-specific covariates. We observed significant associations for a few individual pesticides (for both ever-use and lifetime days of use) and NB outcomes. Ethoprop was significantly associated with adverse performance on a test of motor speed and visual scanning. Malathion was significantly associated with adverse performance on a test of visual scanning and processing. The age-equivalent effect of these two pesticides ranged from 2 to 5 years. Conversely, we observed a dose-response relationship for three OP pesticides significantly associated with better test performance; chlorpyrifos was associated with better motor coordination; chlorpyrifos, coumaphos and tetrachlorvinphos were associated with better verbal learning and

memory. The age-equivalent effect of these three pesticides ranged from 2 to 7 years of age. We also observed a significant interaction by state for a few pesticides and NB outcomes. Although we did see some suggestion of an adverse association with ethoprop and malathion, overall, our results do not provide strong evidence that long-term OP pesticide use is associated with adverse NB test performance among this older sample of pesticide applicators. Reasons for these mostly null associations include a true absence of an association as well as possible selective survival among study members.

Introduction

Organophosphate (OP) pesticides are widely used in the United States and internationally to protect crops from insect damage. According to the U.S. Environmental Protection Agency (EPA), in 2001, OP pesticides accounted for approximately 70% of all insecticide pesticides used in the U.S. with over 73 million pounds used annually [2] . Given the extensive use of these insecticides, exposure is common among agricultural workers as well as the general population and the potential for adverse health outcomes is considerable.

The acute toxicity of OP pesticides on the nervous system is well described and results from the inhibition of the enzyme acetylcholinesterase (AChE). Sufficient inhibition of AChE leads to the accumulation of the neurotransmitter acetylcholine (ACh) and excessive cholinergic activity [4]. Symptoms of acute poisoning generally occur within minutes to hours, depending on the route of exposure.

Long-term exposure to low or moderate-levels of OP pesticides does not cause overt signs of cholinergic toxicity [7, 45]. There is inconsistent evidence, however, that long-term exposure to OPs may cause impaired neurobehavioral function and other adverse neurological outcomes [25, 27, 29-30, 36, 46-47]. The heterogeneity of findings reported in the literature may be due to a number of methodological limitations. One

important limitation of previous studies is small samples with fewer than 50 exposed individuals [24-25, 48]. Studies with small sample sizes result in low statistical power and imprecise estimation of exposure-response relationships. Another limitation of prior studies is the use of poor or inaccurate exposure estimates. Many studies have used dichotomized exposure measures such as job title or occupational classification as surrogate estimates of exposure [27, 29, 35, 46-47]. Such crude exposure estimation methods result in pooling of a wide range of exposures into a few categories with attendant loss of information and reduced statistical power. Previous studies have also used referent groups that may have differed from the exposure group on characteristics other than exposure (e.g., sheep dippers versus ceramic workers) [24-25, 27, 36, 48]. Furthermore, another limitation of the existing literature is inadequate control for potential confounding, such as previous pesticide poisoning [25, 27, 36, 49].

The purpose of this investigation was to examine associations between estimates of cumulative lifetime exposure to OP pesticides and measures of central nervous system (CNS) function in a large cohort of pesticide applicators with well characterized lifetime exposure to OP pesticides. The primary hypothesis to be tested was whether long-term OP pesticide use was associated with adverse neurobehavioral outcomes.

Materials and Methods

We conducted a cross-sectional epidemiological study of the association between neurobehavioral and neurological function and long-term pesticide use among participants enrolled in the Agricultural Health Study (AHS). The AHS is a large, prospective study of licensed pesticide applicators from Iowa and North Carolina [50]. In 1993-1997, 52,395 private applicators enrolled in the AHS by completing a self-administered enrollment questionnaire at the time of pesticide licensing and recertification. A take-home questionnaire completed within one month after enrollment

and two, five-year follow-up phone interviews were administered to AHS participants. The most recent questionnaire was administered within a year of participation in the present study. Information was collected on demographic characteristics, pesticide use information, pesticide application methods, use of personal protective equipment, occupational exposure to other toxicants, and other activities that may influence exposure or disease risk. Copies of AHS questionnaires are available online [51].

Study Participants

Private pesticide applicators who completed all of the AHS questionnaires, resided in Iowa or North Carolina, and lived within approximately 150 miles of the testing facilities were selected for participation in the present study; the same number of participants were selected in each state. Participants were excluded with the following health conditions: amyotrophic lateral sclerosis, multiple sclerosis, Parkinson's disease, retinal or macular degeneration, stroke, hypothyroidism and treatment for diabetes. In addition, participants were excluded who reported drinking $\geq$ 42 alcoholic beverages per week during the most recent AHS interview. Individuals who reported being previously diagnosed with acute pesticide poisoning during this interview were also excluded. To generalize to a population of agricultural pesticide users, the sample was limited to participants who were farming at the time of AHS enrollment. Women were also excluded because they represented less than 1% of licensed pesticide applicators in the AHS cohort. After the eligibility criteria were applied, 1,807 male AHS participants were initially eligible to participate in the present study.

To oversample those individuals with higher lifetime use of OP pesticides, a summary measure of lifetime days of use of all OPs for each applicator was created using AHS enrollment data. The 75th percentile was used as a cut point to create a high and low OP exposure group. Using these two groups, our goal was to achieve a sample with equal numbers from the top 25% of lifetime days of OP use and from the remaining portion of

the sample. Equal numbers of individuals were selected randomly from these two groups and invited to participate. In Iowa, testing was conducted in Iowa City and Dubuque between November 2006 and March 2007. In North Carolina, testing was conducted in Greenville and Wilmington between January 2008 and March 2008. Participants were reimbursed between $100-$175 for time and travel expenses. Appropriate Institutional Review Boards approved the study protocol, and all participants provided written informed consent.

Exposure Assessment

Use of specific pesticides was quantified for each participant using information from all three AHS questionnaires and a health history questionnaire administered at the time of neurobehavioral testing. The AHS questionnaires provided detailed information on 50 commonly used OP and non-OP pesticides, including ever mix or apply, duration of use (years), and frequency of pesticide use (days/year) for each participant. The health history questionnaire provided pesticide use information for the past 12 months including ever mix or apply and duration of use (days).

For OP use metrics, we used the data on the OP pesticides reported on the AHS enrollment questionnaire (chlorpyrifos, coumaphos, diazinon, dichlorvos, fonofos, malathion, parathion, phorate, phosmet and terbufos), the take home questionnaire (acephate, ethoprop, dimethoate, disulfoton and tetrachlorvinphos), and for a new chemical reported on the two follow-up phone interviews (tebupirimfos). Cumulative lifetime days of use was calculated for each pesticide for each participant by multiplying duration of use (years) by frequency of pesticide use (days/year) and adding the number of days used in the past 12 months. We excluded OPs that were used by fewer than 50 people resulting in detailed OP use information for 16 chemicals over the lifetime of the participants. A dichotomized pesticide exposure variable, ever-use, was also created for each pesticide for each participant. In addition to ever-use of specific OP pesticides and

lifetime days of use of specific OP pesticides, a summary variable of OP pesticide use (cumulative lifetime days of all OP pesticides) was created for each participant.

We also obtained information on lifetime days of use of four carbamate pesticides (aldicarb, benomyl, carbaryl and carbofuran) from AHS questionnaires. Two exposure metrics of carbamate pesticide use were created from this information: ever-use and cumulative lifetime days of use.

From pesticide use information collected on AHS questionnaires and the health history questionnaire, a cumulative lifetime days of all pesticide use variable was created. This variable included the 50 commonly used pesticides (both OPs and non-OPs) reported on the AHS enrollment questionnaire. In summary, for every participant, exposure was characterized as: 1) ever-use of each of the 16 OP pesticides; 2) cumulative lifetime days of use of each of the 16 OP pesticides; 3) ever-use of each of the four carbamate pesticides; 4) cumulative lifetime days of use of each of the four carbamate pesticides; 5) cumulative lifetime days of *all* OP pesticide use; and 6) cumulative lifetime days of *all* pesticide use.

Neurobehavioral Testing

Neurobehavioral testing was performed on all participants in private rooms by trained technicians "blinded" to the participants' exposure status. Eight computerized tests from the Neurobehavioral Evaluation System (Version 3) were administered [52-54]. In addition, the manually-administered Grooved Pegboard test was used. These nine tests were selected to be sensitive indicators of a wide range of CNS functions and are briefly described below:

Continuous Performance Test (CPT). The CPT test was used to assess sustained attention. The participant was instructed to press the space bar on a computer keyboard (Dell, Model SK-8135) as quickly as possible when the letter "S" appeared on screen, but not when any other letter appeared. A new letter appeared every second for a five-minute

duration. The summary measure was the mean reaction time in milliseconds for responding to the letter "S". A higher score indicated poorer test performance.

Digit-Symbol Test. The Digit-Symbol test is a modification of a commonly used test from the Wechsler Adult Intelligence Scale [55]. It measured visual scanning and information-processing speed. The test consisted of nine digit-symbol pairs displayed vertically across the top of a touch-screen equipped computer monitor (Elo Touchsystems, Menlo Park, CA) and a row of nine symbols displayed at the bottom of the screen. A random integer (1 to 9) appeared in the middle of the screen. The participant's task was to touch the symbol at the bottom of the screen that was paired with the number as quickly as possible. The summary measure was the latency in seconds to complete responses to 36 items. A higher score indicated poorer test performance.

Finger Tapping. The Finger Tapping test was used to measure manual motor speed and dexterity. Using the index finger of the dominant hand, the participant was instructed to press a computer keyboard's space-bar as many times as possible until instructed to stop. A practice trial was administered followed by four, 10-second trials. The summary measure was the total number of finger taps for the four trials. The test was repeated using the non-dominant hand. A lower score indicated poorer test performance.

Grooved Pegboard. The manually-administered Grooved Pegboard test was used to measure dexterity and fine motor coordination. The Grooved Pegboard Test (Lafayette Instruments, Lafayette, IN) consisted of a metal board with 25 holes with randomly positioned slots and 25 notched pegs. Using the dominant hand, the participant's task was to insert the pegs into the slots in sequence, as quickly as possible. The test was completed when all pegs were placed or after three minutes. The summary measure was the time required in seconds to place all of the pegs. The test was repeated using the non-dominant hand. The maximum score was 180 seconds, and a higher score indicated poorer test performance.

Hopkins Verbal Learning Test (HVLT) Total Recall. HVLT Total Recall was used to assess verbal learning and memory [56]. At the beginning of the test, a list of 12 words was read aloud by the test administrator. The participant was instructed to repeat verbally as many of the words as he could remember. The number correct was recorded by the examiner. Three trials were administered using an identical word list. The summary measure was the total number of correct responses for the three trials. Possible scores ranged from 0 to 36 with a lower score indicating poorer performance.

HVLT Delayed Recall. HVLT Delayed Recall assessed memory and was administered approximately 20 minutes following the HVLT Total Recall trials. The participant was instructed to recall as many words as possible from the original 12-word list. The summary measure was the total number of correct responses and possible scores ranged from 0 to 12. A lower score indicated poorer test performance.

HVLT Recognition. HVLT Recognition was used to assess memory and was administered following the HVLT Delayed Recall test. This test consisted of a 24-word list that included the original 12 words and 12 "distractor" words in random order. The words were read aloud by the test administrator and the participants' task was to correctly identify the words that were included on the original list. The summary measure was the "discrimination index" defined as the number of true positives minus the number of false positives. Possible scores ranged from -12 to 12 with a lower score indicating poorer test performance.

Sequences A. Sequences A is a test of motor speed and tracking. Circles containing the letters "A" through "U" were displayed on the computer screen without special order. The participant was instructed to touch the circles on the monitor in alphabetic order on the touch screen monitor as quickly as possible without making any mistakes. The summary measure was the number of seconds to complete the sequence correctly. A higher score indicated poorer test performance.

Sequences B. Sequences B is also a test of motor speed and tracking and was administered following the Sequences A test. This test required that the participant alternate between number and letter sequences. The participant was instructed to touch the circles alternating between numbers and letters (i.e. 1, A, 2, B, 3, C, etc.). Following the practice trial, a test was administered which consisted of circles labeled "1" through "11" and "A" through "J". The participant was instructed to touch the circles in order, as quickly as possible, without making any mistakes. The summary measure was the number of seconds to complete the sequence correctly. A higher score indicated poorer test performance.

Cognitive Function Summary Measure

Using established methods, a dichotomized summary measure of CNS function was created from Digit-Symbol, Sequences B and Finger Tapping test scores [57]. First, results from the three tests were standardized and the standard scores summed. The summed score was then dichotomized into impaired ($\geq 80^{th}$ percentile) and non-impaired ($<80^{th}$ percentile) groups.

Assessment of Potential Confounders

Potential confounding variables were considered *a priori* and were obtained from several sources. Using existing AHS data and the health history questionnaire administered on the day of neurobehavioral testing, information was obtained on age, height, education, state, smoking status, alcohol consumption, head injury, current antidepressant use, caffeine consumption, and exposure to other potentially neurotoxic substances such as organic solvents, soldering and welding fumes. NES3 Adult Reading Test (ART) scores were measured to estimate premorbid intelligence levels [58]. The summary measure for the NES3 ART was the total number of words pronounced correctly out of 64 words of increasing difficulty displayed on the computer monitor.

Positive and negative affectivity was measured using the NES3 administered Positive and Negative Affect Schedule (PANAS) [59]. Participants were asked 20 questions pertaining to ways they felt "in general". A score of 1-5 was given for each response. The primary measure was the mean score for positive affect and negative affect with higher scores indicating higher affectivity level. Visual acuity was measured using a standard testing instrument, the Optec 1000 (Stereo Optical Co, Chicago, IL). Possible visual acuity scores ranged from 20/20 to 20/200. Scores of 20/50 to 20/200 were considered indicators of poorer visual acuity.

Statistical Methods

Participants' data were excluded from the analysis for the following reasons: alcohol consumption on the day of testing, past diagnosis of alcoholism, brain tumor, macular degeneration, current use of anticonvulsant or psychiatric medication with known cognitive impairment (e.g. benzodiazepines), and renal failure requiring dialysis. Results were excluded for one participant with severe dementia who was unable to understand neurobehavioral testing instructions. Results were also excluded for one participant who reported being struck directly by lightning. In addition, a small number of participants were excluded after standard linear regression diagnostics were performed. Specifically, two subjects were dropped from Digit-Symbol, two from Continuous Performance Test, one from Sequences A and one subject from Sequences B models. These observations were found to be extreme outliers from the overall sample and each had a studentized residual value that exceeded the absolute value of 4.0. These exclusion criteria were applied without reference to exposure information or neurobehavioral testing results.

Linear regression analyses. Analyses began with the creation of a base model for each neurobehavioral outcome with an outcome-specific set of covariates. To examine the association between each covariate and each continuous outcome, unadjusted linear

regression analyses were performed. Those covariates associated with a neurobehavioral outcome with a p-value <0.20 were selected for inclusion in an initial full multiple linear regression base model for that outcome. Covariates with p-values $\geq$ 0.20 were removed sequentially from the initial full base model. The final multivariate base model for each neurobehavioral outcome included only those covariates with p-values <0.20.

The lifetime days of pesticide use variables were log-transformed to normalize the distribution and meet linear regression assumptions. Each pesticide was examined both as a continuous variable (cumulative lifetime days of use) and as a dichotomized variable (ever/never use). Adjusted associations between neurobehavioral outcomes and pesticide exposures were estimated with linear regression models in which the neurobehavioral outcome was regressed on the pesticide exposure variable while controlling for the covariates included in the base model. Parameter estimates for the timed tests (Continuous Performance Test, Digit-Symbol, Grooved Pegboard, Sequences A and B) were inverted so that lower scores indicated poorer test performance for all neurobehavioral outcomes. To compare pesticide age-equivalent effect sizes across the neurobehavioral outcome measures, each adjusted pesticide parameter estimate was converted into an age-equivalent value by dividing it by the base model parameter estimate for age.

In addition, pesticide use by state was examined with the inclusion of a state by pesticide interaction term. Standard linear regression diagnostics were performed on all models; regression diagnostics included studentized residual plots and checks for leverage and influence [60]. Extreme observations were identified and examined for plausibility.

Logistic regression analyses. Logistic regression was used to evaluate the association between pesticide use and a dichotomized cognitive function summary variable. A base model was developed using goodness-of-fit tests and consisted of age, positive affect, adult reading test score, state and visual acuity. Adjusted models were run

with each individual pesticide parameterized as ever-never use with never-users as the referent group. Exposure response was also examined by creating a three-level variable for each pesticide with the distribution of lifetime days of use split at the median among those who had ever used the pesticide. For the pesticide summary variables (lifetime days of all pesticides and lifetime days of all OP pesticides), the distribution was split in quartiles with the lowest exposure category as the referent group. Analyses were restricted to pesticides with more than five exposed cases.

Confounding by related pesticide exposures. Pesticide applicators typically use more than one pesticide. Therefore, the association between the neurobehavioral outcome and any one pesticide may be confounded by one or more other pesticides. Potential confounding of the association between neurobehavioral outcomes and each pesticide by other pesticides was examined for both linear and logistic regression models. Specifically, Spearman correlations were calculated for pesticides associated with neurobehavioral outcomes with a p- value <0.10. Moderately correlated pesticide pairs ($r \geq 0.30$) were added simultaneously to final base models and the pesticide variable parameter estimates were compared to models with only one pesticide. The addition of correlated pesticides to the models did not attenuate any statistically significant associations between the pesticide exposures and the neurobehavioral outcome measures.

Sensitivity analyses. Individuals who reported being diagnosed with acute pesticide poisoning during the most recent AHS interview were excluded from the analyses. However, we did not exclude participants who reported poisoning during earlier interviews. In order to evaluate whether any significant associations between pesticide use and adverse neurobehavioral outcomes were related to previous pesticide poisoning, participants who reported ever being diagnosed with pesticide poisoning were excluded from the analyses and parameter estimates were compared to estimates from models that included the pesticide poisoned individuals.

We used the P1RE1071201, P2RE1071202 and 07222008 releases of the AHS dataset and all analyses were performed using SAS software, version 9.2 (SAS Institute Inc., Cary, NC).

Results

Participation

Among the 1,807 eligible participants, 953 (53%) refused to participate when contacted by telephone and an additional 95 (5%) could not be reached after ten attempts (Figure 2.1). Forty-two percent of those eligible agreed to participate in the study and were scheduled for neurobehavioral testing. Of the 759 participants scheduled for testing, 58 participants cancelled or failed to show for their scheduled appointment. Neurobehavioral testing was administered to 701 participants resulting in an overall response rate of 39 percent.

Thirty-five (5%) participants were excluded from the statistical analyses because of medical conditions, medications (e.g. benzodiazepines) and other factors that may affect neurobehavioral function (Figure 2.1). This resulted in 666 participants available for inclusion in the analyses. These individuals were similar to the 35 excluded individuals by state, smoking history, weekly alcohol consumption, and cumulative lifetime days to all pesticides (data not shown). Participants were however, slightly younger in years than those excluded from the analysis (mean = 61 vs. 64) and were more likely to have completed greater than a high school education (50% vs. 40%) than those excluded from the analyses.

Characteristics of the Study Participants

Demographics. Descriptive statistics for demographic characteristics, personal health information and chemical exposures are presented in Table 2.1. Among the 666

participants included in the analyses, 51% were from Iowa and 49% were from North Carolina. The mean age of the participants was 61 years (SD = 12) and half reported completing at least a high school education. Over 20% of the participants reported a past head injury with or without loss of consciousness and eight (1%) reported a previous diagnosis of acute pesticide poisoning.

Pesticide exposures. Frequencies of specific pesticide use and means of cumulative lifetime days for the 16 OPs, four carbamates and two pesticide summary variables are presented in Table 2.2. OP use ranged from 78% for malathion to 10% for dimethoate, tebupirimfos and tetrachlorvinphos. Carbaryl (63%) was most commonly used among the carbamate pesticides. Most participants reported ever using any OP (98%) and all but one participant reported using at least one pesticide in their lifetime. Lifetime days of all OP pesticides and lifetime days of all pesticides were similar between Iowa and North Carolina participants (data not shown). Carbamate pesticide use, however, was more prevalent in North Carolina (93%) than in Iowa (67%).

Neurobehavioral outcome measures. Descriptive summary statistics for the neurobehavioral test results are presented in Table 2.3. Finger Tapping and Grooved Pegboard were administered separately for the dominant and non-dominant hands. However, because the overall results were similar for both hands, only the results for the dominant hand are presented. The total number of participants completing each test varied because some study participants were unable to complete the test in the allowed time or after two attempts, or because of computer problems or test administrator error. (Comparative values for neurobehavioral tests are presented in Appendix A).

Linear Regression Base Model Covariates

Each base model included the neurobehavioral outcome of interest and all relevant covariates (Table 2.4). Age and Adult Reading Test (ART) scores were significant covariates for all neurobehavioral measures at a p-value <0.01. State was

included for all outcomes except the Continuous Performance Test and HVLT Total Recall. The total variance (r^2) accounted for by the regression models ranged from 0.16 for Finger Tapping to 0.48 for the Digit-Symbol Test.

Associations between OP pesticide Use and Neurobehavioral Outcomes

Lifetime days of all OP use was not associated in either direction with neurobehavioral function. Specific pesticide use was associated with some but not all of the tests. Four of the nine continuous outcomes we examined had at least one significant adverse association with ever-use (Table 2.5) or lifetime days of pesticide use (Table 2.6). Ethoprop and malathion were both significantly associated with poorer performance on the Digit-Symbol test. Conversely, five of the nine outcomes had significantly better test performance with ever-use or lifetime days of use. Better test performance was observed more frequently for the three Hopkins Verbal Learning tests (Total Recall, Delayed Recall and Recognition). For several neurobehavioral outcomes, we observed a significant state by pesticide interaction, suggesting differential effects for chlorpyrifos, coumaphos, malathion and cumulative lifetime days of use of all OPs in North Carolina and Iowa (Table 2.7). Although we did see some suggestion of an adverse association with a few chemicals, overall, our results do not provide strong evidence that long-term OP pesticide use is associated with adverse NB test performance as discussed below.

Continuous Performance Test (CPT). Participants reporting ever using malathion were significantly slower on CPT than those who never used malathion; however there was no evidence of a dose response relationship. This effect size is equivalent to 4.9 years of age in this population. No significant associations were observed with CPT and other pesticide exposures.

Digit-Symbol. Ever-use and lifetime days of malathion use were both significantly associated with poorer Digit-Symbol test performance. Significant adverse associations

were also observed with ever-use of ethoprop. The age equivalent effect sizes for these two chemicals range from 2.0 to 3.8 years. When an interaction term for state by pesticide was included in the models, the association between chlorpyrifos and Digit-Symbol test performance differed by state. In Iowa, ever-use and lifetime days of chlorpyrifos use was significantly associated with poorer test performance, whereas in North Carolina we observed better, but non-statistically significant test performance.

Grooved Pegboard. Ever-use and lifetime days of chlorpyrifos use was significantly associated with better Grooved Pegboard test performance. These effect sizes were equivalent to -3.4 and -1.9 years of age. A significant interaction between state and malathion (both ever-use and lifetime days of use) was also observed. North Carolina participants who reported ever using malathion had significantly poorer test performance, whereas better, but non-statistically significant, test performance was observed in Iowa.

Hopkins Verbal Learning Tests (HVLT). Lifetime days of ethoprop use was significantly associated with poorer performance on the Total Recall test. Conversely, ever using chlorpyrifos, coumaphos and tretrachlorvinphos were significantly associated with better Total Recall and Delayed Recall test performance. Lifetime days of use of five OP pesticides (coumaphos, chlorpyrifos, parathion, phorate and tetrachlorvinphos) were significantly associated with better test performance on at least one of the three HVLT tests. Age-equivalent effect sizes for these pesticides ranged from -2.7 to -6.6 years. When an interaction term for state by pesticide was included in the HVLT Total Recall models, we observed a significant interaction between state and lifetime days of all OP pesticides. Specifically, among Iowa participants, lifetime days of all OP pesticide use was significantly associated with better Total Recall test performance, whereas poorer, but non-statistically significant, test performance was observed among North Carolina participants. Additionally, in the HVLT recognition models, lifetime days of

coumaphos use was significantly associated with better test performance among North Carolina participants, while no association was observed among Iowa participants.

Cognitive function summary measure. Consistent with the results from the linear regression analyses, ethoprop and malathion were associated with poorer neurobehavioral function: ever-use of ethoprop (odds ratio (OR) = 1.79, 95% confidence interval (CI): 1.01, 3.17) and malathion (OR = 1.75, 95% CI: 1.00, 3.07). There was also evidence of a dose-response trend for ethoprop (p=0.04) and malathion (p=0.06) with the largest ORs at the highest exposure level.

Associations between Carbamate Use and Neurobehavioral Outcomes

No statistically significant associations were observed between carbamate use and adverse neurobehavioral outcomes. Rather, all four carbamate pesticides were significantly associated with better performance on one or more neurobehavioral test (Tables 2.5-2.6). The age-equivalent effect sizes for these chemicals ranged from -3.4 to -6.0 years. A significant interaction between state and ever-use of carbofuran was observed for Sequences B (Table 2.7). Among Iowa participants, better test performance was significantly associated with ever-use, whereas poorer, but non-statistically significant test performance was observed among North Carolina participants.

Sensitivity Analyses

In order to evaluate whether the present results were biased by previous pesticide poisoning, the eight participants who reported physician diagnosed pesticide poisoning were excluded and the analyses were rerun. When poisoned individuals were removed from the analysis, the parameter estimate of the association between ever ethoprop use and Digit-Symbol test performance was attenuated from 4.04 to 3.55 seconds, but

remained statistically significant. All other associations were unaffected by the exclusion of participants with previous pesticide poisoning.

Discussion

The current study was designed to examine associations between long-term pesticide use and neurobehavioral outcomes. The results indicate that ever-use and lifetime days of pesticide use were associated with significantly poorer performance on four of nine neurobehavioral tests and significantly better performance on five of nine tests. This is among the largest studies of neurobehavioral function among pesticide-exposed workers published to date; we had good characterization of specific pesticide exposure and formal measures of neurobehavioral function, however, little evidence of an adverse association with pesticide use was observed.

The authors of several previous studies of agricultural workers reported poorer neurobehavioral function among those exposed to pesticides (in the absence of previous pesticide poisoning). Rohlman et al administered a battery of 10 neurobehavioral tests to 119 Hispanic adults and adolescents working in agriculture and 56 Hispanic adults and adolescents not working in agriculture [47]. The mean age of the adults working in agriculture was 28.2 years (SD = 7.6). Statistically significantly poorer test performance was observed on four neurobehavioral measures (including the Continuous Performance Test, as found in the current study) among those with any experience mixing or applying pesticides. Although not statistically significant, Rohlman et al also observed an adverse association between mixing or applying pesticides and the Digit-Symbol test. Farahat et al studied 52 male workers occupationally exposed to OP pesticides and 50 unexposed male controls with similar demographic characteristics [27]. The mean age of the workers was 44 years (SD=5.5). After adjustment for age and education, workers occupationally exposed to OP pesticides performed significantly poorer than unexposed workers for six

of 12 neurobehavioral tests, including Digit-Symbol and Trailmaking part A and B (similar to Sequences A and B in the current study). Kamel et al conducted a cross-sectional study of neurobehavioral test performance among 288 farm workers with at least one month of farm work exposure and 51 controls without farm work exposure [46]. The mean age of the farmworkers was 40 years (SD = 7.3) "Ever having done farm work" was associated with poorer performance on four of eight neurobehavioral tests including tests of verbal memory, motor speed and motor coordination. Adverse associations were observed in the absence of 19 individuals with a history of acute pesticide poisoning.

Contrary to much of the published literature, we observed significantly better performance on several neurobehavioral tests among pesticide users. Specifically, better performance was observed for OP and carbamate pesticides and tests of verbal learning and memory (HVLT Total Recall, Delayed Recall and Recognition). For example, significant associations were observed between HVLT Delayed Recall and cumulative lifetime days of use for two of the 16 OP pesticides, three of the four carbamate pesticides, and cumulative lifetime days of use of all pesticides. A few previous studies have also reported significantly better test performance among pesticide exposed subjects [19, 22]. However, in contrast to the present study, these studies did not observe significant positive associations for tests of verbal learning or memory. Given the consistency of improved performance on tests of verbal learning and memory and use of OP and carbamate pesticides, further investigation may be warranted.

Several statistically significant interactions were observed between state and indices of pesticide exposure. The explanation for these interactions is unclear. However, the difference in findings observed between Iowa and North Carolina participants may be due to differences in pesticide use and application methods. For example, chlorpyrifos, one of the most widely used pesticides in both states, is more commonly used in a granular formulation in Iowa and a liquid formulation in North Carolina. In a recent AHS

study, Thomas et al found higher urinary levels of a chlorpyrifos metabolite (TCP) in individuals performing spray applications of liquid chlorpyrifos products compared to individuals who apply granular chlorpyrifos products [61]. These differences may influence individual exposure to these pesticides in a manner not captured by the exposure metric used in the present study.

This study has several limitations that may affect inferences made from the results. First, it is likely that our sample was highly selected. Although we randomly sampled from the AHS cohort, we required individuals to complete all AHS questionnaires and excluded individuals with a number of health conditions. It is possible that individuals who left farming or who were unable to complete the AHS questionnaires may have been more affected by pesticides than those who were eligible and participated in the current study. Furthermore, the average age of our population was older compared with most previous studies. An older cohort is more likely to manifest selective survival than a younger cohort. This potential selection bias may have attenuated the observed associations between long-term pesticide use and neurobehavioral outcomes.

Second, the overall response rate of the study was less than 40% which suggests that our study sample may not have been representative of pesticide applicators enrolled in the AHS. However, participants were similar to those who did not participate on several important characteristics including age and total lifetime days of pesticide use, suggesting comparability between participants and non-participants.

Another potential limitation of this study is the accuracy of self-reported pesticide use and practices and other information such as health conditions and health-related behaviors (e.g. smoking, alcohol consumption, medication use). Substantial misclassification of pesticide use is unlikely since methodologic studies have shown that AHS participants provide accurate and reliable pesticide use and duration of pesticide exposure information [62-64]. Using pesticide registration information, Hoppin et al

showed that AHS participants provide plausible data regarding lifetime duration of use, with less than 5% reporting implausible values for specific chemicals [64]. Similar findings were reported by Blair et al who reported that for repeated interviews the percentage agreement for specific pesticide use and application practices were high, ranging from 70% to more than 90% [63].

Our study assessed multiple chemicals and multiple outcomes. Given that we performed over 700 statistical tests and observed only 35 significant findings in both positive and negative directions, we cannot rule out the possibility that some of our findings may be due to chance. It is unlikely, however, that the relatively consistent findings on tests of verbal learning and memory occurred by chance alone. Rather, these results may be attributable to bias introduced by selective survival or other factors.

A major strength of the study is that it was based on a large sample of pesticide applicators selected from the AHS. The sample included pesticide applicators from two distinct geographical locations with different crops and farming practices. Therefore, the results of the present study are likely relevant to a large segment of the farming population. Unlike many prior studies, we had sufficient power to examine the associations of individual pesticides with neurobehavioral outcomes while controlling for important covariates. However, we were not well powered for examination of interactions, so for those associations that differed between states or for those pesticides used in only one state, we had limited power.

Another important strength of the study is the use of relatively precise exposure estimates. Detailed information on pesticide use has been periodically updated by the AHS since 1993 and represents true prospective exposure information. Whereas most studies in the literature used dichotomized exposure variables, we estimated cumulative lifetime days of use to specific OP pesticides, as well as to this class as a whole, for each study participant. Furthermore, we were able to explore possible dose-response

relationships between each pesticide and the neurobehavioral outcomes by examining associations between lifetime days of pesticide use and neurobehavioral outcomes.

Participants in the present study completed neurobehavioral testing during the winter months (November - March) when pesticide application was minimal. Therefore, it is unlikely that the observed adverse associations are the result of an acute cholinergic response to recent pesticide exposure. Furthermore, participants who reported a past diagnosis of pesticide poisoning at the most recent AHS interview were excluded from the sample. In a sensitivity analysis, eight participants who reported previously diagnosed pesticide poisoning at the time of AHS enrollment (but not the most recent AHS interview), were excluded from the statistical analysis. The exclusion of these individuals did not affect the results. In light of these results, it is unlikely the adverse associations observed in this study are the result of long-term sequelae of pesticide poisoning.

In conclusion, we did not observe strong evidence of adverse neurobehavioral outcomes in this large neurobehavioral study of licensed pesticide applicators. While our results may be due to chance, some of the findings are consistent with previous studies. Additionally, for some pesticides we observed differential associations by state, suggesting that some aspect of pesticide use may influence neurotoxicity, but we were underpowered to evaluate state by pesticide interactions thoroughly.

Figure 2.1. Non-participation and reasons for exclusion

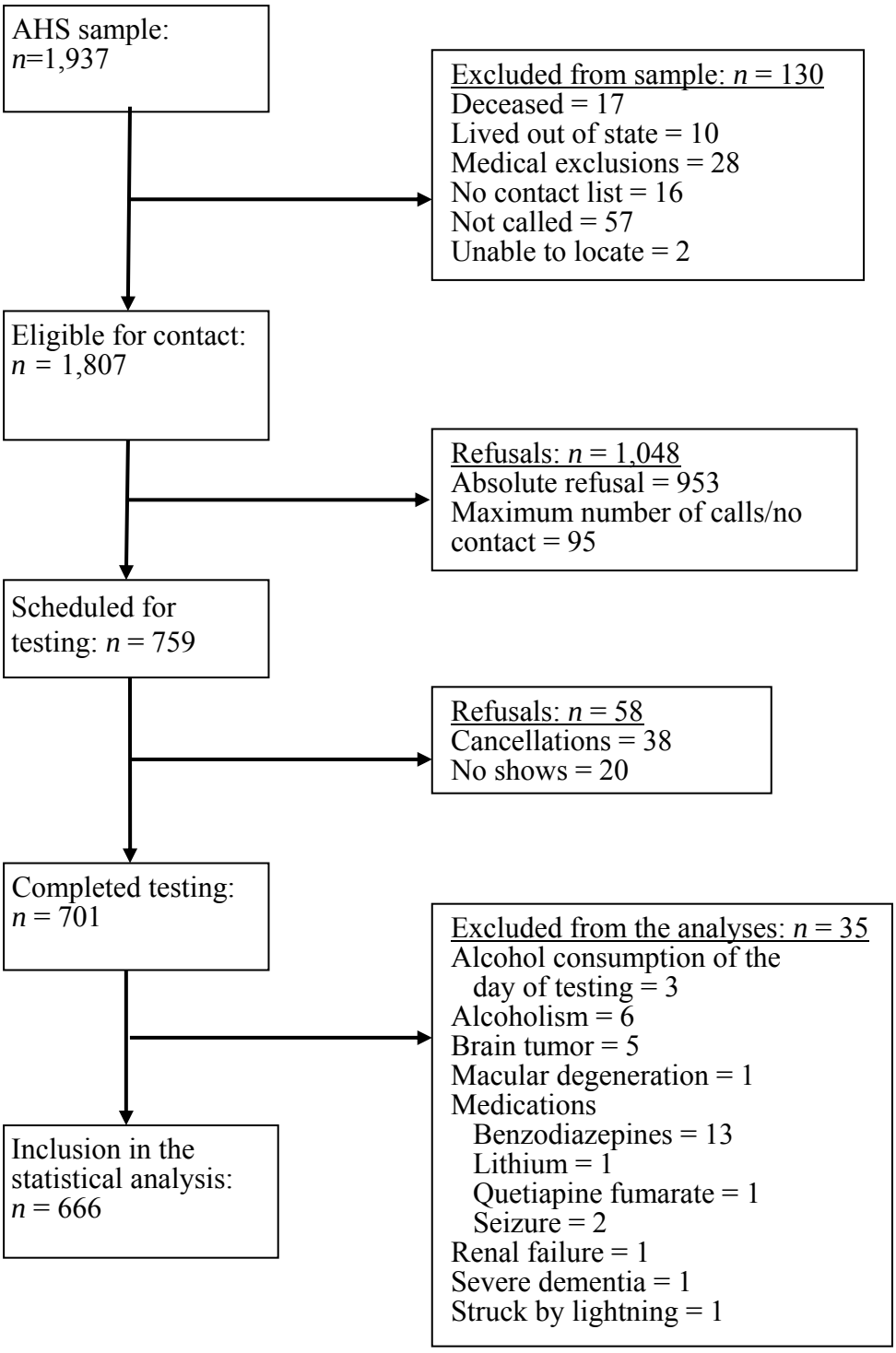

Table 2.1. Demographic characteristics, personal health information and chemical exposure (n=666)

Characteristic	Mean	SD	No.	%
Age (yrs)	61	12	--	--
Height (cm)	179	7	--	--
Adult Reading Test (0-60)	30	10	--	--
Positive affect (1-5)	3.5	0.7	--	--
Negative affect (1-5)	1.4	0.4	--	--
Testing location				
Iowa	--	--	340	51
North Carolina	--	--	326	49
Education				
$\leq$ High school	--	--	334	50
> High school	--	--	332	50
Smoking status				
Never smoked	--	--	383	58
Current smoker	--	--	43	6
Past smoker	--	--	240	36
Alcohol consumption (drinks/wk)				
0 drinks	--	--	380	57
1-7 drinks	--	--	223	34
>7 drinks	--	--	63	9
Visual acuity				
20/20 - 20/40	--	--	566	85
20/50 - 20/200	--	--	100	15
Head injury				
No injury	--	--	510	77
Injury, no loss of consciousness	--	--	69	10
Injury, w/loss of consciousness	--	--	87	13
Antidepressants (current use)	--	--	42	6
Caffeine use (drink regularly)	--	--	499	75
Solvent exposure (ever)	--	--	279	42
Soldering exposure (ever)	--	--	32	5
High pesticide exposure event (ever)	--	--	163	24
Pesticide poisoning (ever)	--	--	8	1

Table 2.2. Frequencies and means of cumulative lifetime days of pesticide use (n=666)

Pesticides	N*	%	Mean	SD	Min.	Median	Max.
Organophosphates							
Acephate	158	24	88	92	3	56	501
Chlorpyrifos	406	61	69	103	2	29	767
Coumaphos	92	14	59	183	1	10	1,628
Diazinon	291	44	54	93	1	20	846
Dichlorvos	123	18	406	921	1	56	5,880
Dimethoate	64	10	47	69	2	25	457
Disulfoton	106	16	43	42	2	25	236
Ethoprop	115	17	45	50	3	25	316
Fonofos	195	29	64	85	2	39	457
Malathion	518	78	88	197	2	26	2,625
Parathion	141	21	104	276	1	20	1,668
Phorate	217	33	71	138	1	25	1,628
Phosmet	98	15	60	84	3	25	600
Tebupirimfos	65	10	52	46	4	40	250
Terbufos	337	51	105	56	2	56	752
Tetrachlorvinphos	67	10	66	100	3	25	582
Carbamates							
Aldicarb	125	19	82	115	2	26	601
Benomyl	112	17	83	124	1	15	767
Carbaryl	421	63	92	136	1	39	1,238
Carbofuran	275	41	55	92	1	25	752
Summary variables							
All organophosphates	650	98	397	314	2	225	5,959
All pesticides	665	100	1,630	1,642	18	1,069	11,677

* Number of participants who reported ever use

Table 2.3. Frequencies and means of neurobehavioral outcome measures (n=666)

Outcome	N	Mean	SD	Min.	Median	Max.
Continuous performance (ms)	657	426.5	43.8	318.6	420.2	612.3
Digit-Symbol (s)	658	117.3	22.8	73.6	111.7	213.6
Finger tapping, dominant hand (# of taps)	662	53.6	9.6	9.0	55.0	86.0
Grooved pegboard, dominant hand (s)	665	91.3	23.5	51.0	86.0	180.0
Hopkins verbal learning total recall (# correct)	662	20.0	5.0	6.0	20.0	34.0
Hopkins verbal learning delayed recall (# correct)	662	6.7	2.8	0	7.0	12.0
Hopkins verbal learning recognition (tp-fp)	662	8.4	2.6	-3.0	9.0	12.0
Sequences A latency (s)	650	42.8	14.6	14.8	40.2	93.8
Sequences B latency (s)	640	64.1	21.0	22.8	59.5	144.4

NOTE: tp = true positives; fp = false positives

Table 2.4. Base model regression coefficients for neurobehavioral outcome measures (n=666)

Outcome	Age (yrs)	ART score	NA score	PA score	Caffeine	Education	State	Visual acuity score	Base model R^2
CPT[c] (ms)	-1.53[b]	0.60[b]	--	3.25[b]	-6.52	--	--	--	0.22
Digit-Symbol Test[c] (s)	-1.07[b]	0.43[b]	--	4.56[b]	--	3.03[a]	-4.92[b]	-4.79[a]	0.48
Finger Tapping, dominant (# of taps)	-0.24[b]	0.14[b]	--	0.99	--	--	-2.48[a]	--	0.16
Grooved Pegboard, dominant [c] (s)	-1.04[b]	0.14[b]	--	--	-3.35	--	-4.04[b]	-6.93[b]	0.34
HVLT Total Recall (# correct)	-0.18[b]	0.11[b]	-0.89[a]	0.67[b]	--	0.79	--	--	0.28
HVLT Delayed Recall (# correct)	-0.09[b]	0.05[b]	-0.47[a]	0.37[a]	--	0.67[b]	-0.54[b]	--	0.26
HVLT Recognition (tp-fp)	-0.05[b]	0.05[b]	--	--	--	0.69[b]	-0.83[b]	--	0.20
Sequences A[c] (s)	-0.64[b]	0.39[b]	--	2.16[b]	--	--	-2.74[b]	--	0.41
Sequences B[c] (s)	-0.93[b]	0.53[b]	--	4.00[b]	--	--	-4.95[b]	--	0.42

NOTE: ART = Adult reading test; NA = negative affect; PA = positive affect; CPT = Continuous Performance Test; HVLT = Hopkin's Verbal Learning Test; tp= true positives; fp = false positives. Age, ART, NA and PA are continuous variables. Caffeine, education, state and visual acuity are categorical variables. The reference groups for the categorical variables are, caffeine = drink regularly, education = $\leq$ high school education, state = Iowa, visual acuity = 20/20 - 20/40 vision

[a] $p<0.05$; [b] $p<0.01$; [c] Scores have been inverted so that lower scores indicate poorer performance

Table 2.5. Regression coefficients from linear regression models for neurobehavioral outcomes and ever used pesticides

Pesticide	CPT[c] (n=657)		Digit-Symbol[c] (n=658)		Finger Tapping, dominant (n=662)		Grooved Pegboard, dominant[c] (n=665)		HVLT Total Recall (n=662)		HVLT Delayed Recall (n=662)		HVLT Recognition (n=662)		Sequences A[c] (n=650)		Sequences B[c] (n=640)	
	β	SE	β	SE	β	SE	β	SE	β	SE	β	SE	β	SE	β	SE	β	SE
Organophosphates																		
Acephate	0.27	3.59	-2.41	1.87	0.18	0.99	-0.65	2.14	-0.05	0.40	0.24	0.27	-0.06	0.26	-2.18	1.29	-1.59	1.87
Chlorpyrifos	-3.00	3.17	(int)	--	-0.75	0.72	**3.59**[a]	1.54	0.55	0.35	**0.41**[a]	0.20	0.11	0.19	-1.41	0.93	-1.01	1.33
Coumaphos	0.87	4.41	1.93	1.90	1.15	1.01	1.17	2.18	**1.19**[b]	0.48	0.38	0.28	0.36	0.26	-0.86	1.29	2.58	1.84
Diazinon	-1.24	3.10	0.20	1.35	0.82	0.71	0.14	1.55	0.16	0.34	0.20	0.20	-0.04	0.19	-0.81	0.92	0.51	1.33
Dichlorvos	-2.02	3.92	-0.93	1.79	0.03	0.96	0.36	2.06	0.44	0.44	0.06	0.26	0.14	0.25	1.55	1.22	1.06	1.76
Dimethoate	1.01	5.14	-1.82	2.20	-0.82	1.17	-0.82	2.53	0.82	0.57	0.12	0.32	0.54	0.30	1.89	1.49	-1.70	2.15
Disulfoton	1.91	4.19	-1.87	1.96	-1.24	1.05	-2.22	2.25	-0.21	0.47	0.38	0.28	-0.31	0.27	-2.59	1.35	0.28	1.97
Ethoprop	0.84	4.03	**-4.04**[a]	1.88	0.29	1.01	-1.96	2.17	-0.84	0.45	-0.20	0.27	-0.42	0.26	**-3.35**[b]	1.29	-1.72	1.89
Fonofos	1.34	3.36	1.29	1.59	0.09	0.85	0.20	1.82	0.50	0.37	0.34	0.23	0.01	0.22	-0.44	1.09	-0.08	1.56
Malathion	**-7.47**[a]	3.71	**-3.43**[a]	1.58	0.24	0.84	(int)	--	0.46	0.41	0.17	0.23	-0.29	0.22	-0.88	1.09	-0.27	1.56
Parathion	2.32	3.77	-0.11	1.64	-0.20	0.87	0.33	1.87	-0.36	0.41	0.37	0.24	0.01	0.23	-0.89	1.12	-1.47	1.61
Phorate	-1.23	3.25	0.18	1.43	-0.15	0.77	-0.23	1.65	-0.33	0.36	0.28	0.21	0.29	0.20	-0.40	0.98	0.42	1.41
Phosmet	-3.57	4.37	-2.26	1.95	0.38	1.04	0.94	2.25	0.29	0.48	-0.26	0.28	-0.20	0.27	-0.15	1.33	-0.31	1.89
Tebupirimfos	-1.29	5.12	2.42	2.29	0.19	1.22	-1.64	2.65	0.36	0.57	0.30	0.33	-0.05	0.32	-0.65	1.58	1.66	2.24
Terbufos	-0.77	3.05	1.06	1.36	1.30	0.72	-1.22	1.55	0.21	0.34	0.20	0.20	0.07	0.19	-1.69	0.93	-0.29	1.33
Tetrachlorvinphos	2.37	5.04	0.29	2.21	-1.50	1.18	-1.52	2.54	**1.18**[a]	0.56	0.18	0.32	0.50	0.31	1.38	1.50	1.32	2.14
Carbamates																		
Aldicarb	-2.43	3.92	0.72	1.87	-0.58	0.99	2.52	2.13	0.31	0.48	0.35	0.27	0.41	0.26	-0.72	1.28	-0.10	1.85
Benomyl	3.08	4.09	2.04	1.88	-0.47	1.00	-1.08	2.15	0.57	0.48	**0.52**[a]	0.27	0.20	0.26	**2.55**[a]	1.28	-0.97	1.86
Carbaryl	-1.10	3.19	0.01	1.52	(int)	--	-2.04	1.75	**0.79**[a]	0.39	**0.54**[a]	0.22	0.03	0.21	-0.32	1.05	0.49	1.50
Carbofuran	3.76	3.12	1.35	1.34	1.21	0.71	1.98	1.53	0.26	0.35	0.24	0.19	-0.12	0.18	-0.85	0.92	(int)	--

NOTE: HVLT = Hopkin's Verbal Learning Test; int = significant interaction term ($p<0.05$) for state by pesticide exposure. The results for models with interaction by state are presented in Table 7; Models are adjusted for the base model covariates listed in Table 2.4

[a] $p<0.05$; [b] $p<0.01$; [c] Scores have been inverted so that lower scores indicate poorer performance

Table 2.6. Regression coefficients from linear regression models for neurobehavioral outcomes measures and cumulative lifetime days of pesticide use (log10 transformed)

Pesticide	CPT[c] (n=657)		Digit-Symbol[c] (n=658)		Finger Tapping, dominant (n=662)		Grooved Pegboard, dominant[c] (n=665)		HVLT Total Recall (n=662)		HVLT Delayed Recall (n=662)		HVLT Recognition (n=662)		Sequences A[c] (n=650)		Sequences B[c] (n=640)	
	β	SE	β	SE	β	SE	β	SE	β	SE	β	SE	β	SE	β	SE	β	SE
Organophosphates																		
Acephate	0.22	0.91	-1.20	1.02	-0.09	0.54	-0.22	1.16	-0.08	0.22	0.13	0.15	-0.04	0.14	-0.91	0.70	-0.44	1.02
Chlorpyrifos	-0.88	1.81	(int)	--	-0.17	0.41	**1.96**[a]	0.88	0.25	0.20	**0.24**[a]	0.11	0.00	0.11	-0.84	0.53	-0.82	0.76
Coumaphos	1.47	3.15	2.07	1.35	1.03	0.72	1.54	1.56	**0.80**[a]	0.35	0.23	0.20	(int)	--	0.43	0.92	**2.92**[a]	1.32
Diazinon	-0.51	1.99	-0.60	0.88	0.24	0.47	-0.39	1.01	-0.02	0.22	0.04	0.13	-0.03	0.12	-0.29	0.60	0.44	0.87
Dichlorvos	-1.50	1.87	0.02	0.85	-0.17	0.46	-0.30	0.98	0.28	0.21	0.12	0.12	0.13	0.12	0.57	0.58	0.52	0.83
Dimethoate	1.04	3.42	-0.93	1.46	-0.06	0.78	-0.67	1.69	0.37	0.38	0.03	0.21	0.35	0.20	1.33	0.99	-1.36	1.43
Disulfoton	1.98	2.72	-1.37	1.27	-0.77	0.67	-1.26	1.45	-0.25	0.30	0.20	0.18	-0.24	0.18	-1.54	0.87	0.01	1.27
Ethoprop	1.68	2.61	-1.95	1.21	0.03	0.65	-0.83	1.40	**-0.57**[a]	0.29	-0.15	0.18	-0.25	0.17	**-1.68**[a]	0.83	-1.14	1.21
Fonofos	0.59	2.03	0.68	0.95	-0.04	0.51	0.63	1.09	0.36	0.23	0.26	0.14	-0.06	0.13	-0.22	0.65	-0.34	0.93
Malathion	-1.18	1.89	**-2.09**[b]	0.80	0.18	0.43	(int)	--	0.08	0.21	0.07	0.12	-0.10	0.11	-0.45	0.56	0.05	0.80
Parathion	3.75	2.35	0.03	1.01	-0.50	0.54	-0.34	1.16	0.06	0.26	**0.31**[a]	0.15	0.17	0.14	0.14	0.69	-0.42	0.99
Phorate	0.24	1.96	0.79	0.85	-0.32	0.46	-0.09	0.98	0.25	0.22	0.25	0.12	**0.24**[a]	0.12	0.08	0.59	0.41	0.84
Phosmet	-1.83	2.70	-1.28	1.20	0.33	0.64	0.52	1.38	0.11	0.30	-0.23	0.17	-0.11	0.17	-0.07	0.82	-0.15	1.17
Tebupirimfos	-1.33	3.15	1.52	1.41	0.04	0.75	-1.49	1.62	0.32	0.35	0.24	0.20	-0.03	0.20	-0.35	0.97	1.05	1.38
Terbufos	0.05	1.65	0.73	0.73	0.69	0.39	-0.86	0.84	0.11	0.18	0.10	0.11	0.05	0.10	-0.62	0.50	-0.27	0.72
Tetrachlorvinphos	1.78	3.10	0.19	1.35	-0.92	0.73	-0.97	1.56	**0.71**[a]	0.34	0.09	0.20	0.34	0.19	1.17	0.92	0.68	1.31
Carbamates																		
Aldicarb	0.32	2.32	1.01	0.36	-0.18	0.58	1.95	1.26	0.32	0.28	**0.31**[a]	0.16	0.26	0.15	-0.07	0.75	0.69	1.10
Benomyl	2.25	2.72	2.23	1.24	-0.02	0.66	0.15	1.42	**0.66**[a]	0.32	**0.48**[b]	0.18	0.25	0.17	**2.45**[b]	0.84	1.60	1.22
Carbaryl	0.05	1.71	-0.43	0.87	(int)	--	-1.11	1.01	0.11	0.23	0.21	0.13	-0.02	0.12	-0.01	0.60	0.09	0.87
Carbofuran	1.19	2.00	0.56	0.85	0.65	0.45	1.59	0.97	0.25	0.25	**0.25**[a]	0.12	0.03	0.12	-0.50	0.58	(int)	--
Summary variables																		
ALL OPs	-1.72	2.26	-1.14	0.97	-0.37	0.52	-1.15	1.11	(int)	--	0.21	0.14	-0.06	0.13	(int)	--	0.04	0.95
ALL pesticides	0.90	3.20	-1.73	1.37	0.55	0.72	-0.26	1.56	0.42	0.35	**0.54**[b]	0.20	0.04	0.19	-1.45	0.94	-1.45	1.34

NOTE: HVLT = Hopkin's Verbal Learning Test; int = significant interaction term ($p<0.05$) for state by pesticide exposure. The results for models with interaction by state are presented in Table 7; Models are adjusted for the base model covariates listed in Table 2.4

[a] $p<0.05$; [b] $p<0.01$; [c] Scores have been inverted so that lower scores indicate poorer performance

Table 2.7. Regression coefficients from linear regression models for neurobehavioral outcome measures and pesticide exposures with an interaction term for state by pesticide exposure

	Digit-Symbol[c] (n=658)		Finger Tapping (n=662)		Grooved Pegboard[c] (n=658)		HVLT Total Recall (n=662)		HVLT Recognition (n=662)		Sequences A[c] (n=650)		Sequences B[c] (n=640)	
	Iowa	N.C.	Iowa	N.C.	Iowa	N.C.	Iowa	N.C.	Iowa	N.C.	Iowa	N.C.	Iowa	N.C.
Lifetime days (log_{10} transformed)														
Carbaryl	--	--	1.06	-0.93	--	--	--	--	--	--	--	--	--	--
Carbofuran	--	--	--	--	--	--	--	--	--	--	--	--	1.46	-2.04
Chlorpyrifos	**-2.45**[b]	0.65	--	--	--	--	--	--	--	--	--	--	--	--
Coumaphos	--	--	--	--	--	--	--	--	-0.01	**1.09**[b]	--	--	--	--
Malathion	--	--	--	--	1.84	-1.80	--	--	--	--	--	--	--	--
ALL OP pesticides	--	--	--	--	--	--	**0.66**[a]	-0.38	--	--	0.71	**-2.05**[b]	--	--
Ever used														
Carbaryl	--	--	1.35	-1.80	--	--	--	--	--	--	--	--	--	--
Carbofuran	--	--	--	--	--	--	--	--	--	--	--	--	**3.40**[a]	-3.44
Chlorpyrifos	**-4.92**[b]	1.67	--	--	--	--	--	--	--	--	--	--	--	--
Malathion	--	--	--	--	4.61	**-5.50**[a]	--	--	--	--	--	--	--	--

NOTE: CPT = Continuous Performance Test; HVLT = Hopkins Verbal Learning Test; Models are adjusted for the base model covariates listed in Table 2.4 and an interaction term for state by pesticide exposure. Results are presented for models with significant interaction term ($p<0.05$)

[a] $p<0.05$; [b] $p<0.01$; [c] Scores have been inverted so that lower scores indicate poorer performance

CHAPTER III

HIGH PESTICIDE EXPOSURE EVENTS AND CENTRAL NERVOUS SYSTEM FUNCTION IN PESTICIDE APPLICATORS

Abstract

While acute pesticide poisoning is associated with persistent adverse central nervous system (CNS) effects, little is known about the effect of episodic and unusually high pesticide exposure events (HPEEs) that typically do not result in acute poisoning. The results of neurobehavioral (NB) tests administered to licensed pesticide applicators enrolled in the Agricultural Health Study (AHS) were used to examine the association between HPEEs and CNS function. In 2006-2008, 666 male participants completed nine NB tests to assess memory, motor speed, sustained attention, verbal learning, and visual scanning/processing. Information on HPEEs and pesticide poisonings was obtained from previous AHS interviews. A history of at least one HPEE was reported by 155 (23%) participants. Associations between HPEEs and NB outcomes were estimated with linear regression controlling for age and outcome-specific covariates. Adverse associations were observed between HPEEs and two of the nine NB tests. On a test of visual scanning and processing (*Digit-Symbol*), participants with HPEEs were 4.9 seconds slower ($p<0.01$) than those without HPEEs, equivalent to the effect of 4.6 years of age in this population. On a test of motor speed and visual scanning (*Sequences A*), participants with HPEEs were 2.2 seconds slower ($p<0.05$) than those without HPEEs, equivalent to the effect of 3.4 years of age. These results were unaffected by the exclusion of eight participants with past acute pesticide poisoning. No significant associations were observed between HPEEs and the other NB tests. In summary, small but meaningful

associations were observed between HPEEs and adverse CNS outcomes related to visual scanning independent of pesticide poisoning.

Introduction

The acute toxicity of pesticide poisoning on the nervous system and other organs is well described. Mild acute toxic effects include headache, dizziness, nausea, vomiting and diarrhea whereas more severe acute toxicity includes cardiac rhythm disturbances, seizures, respiratory failure and coma. Although mild symptoms often resolve shortly after cessation of exposure, acute pesticide poisoning has been associated with long-term neurological sequelae, including deficits in neurobehavioral (NB) test performance and an increase in neurological symptoms [17, 19, 21, 35, 65].

In addition to the acute and long-term toxicity of overt pesticide poisoning, there is some evidence that prolonged exposure to pesticides at levels insufficient to cause clinical toxicity results in persistent adverse neurological function. However, the evidence is limited. Not all high-level pesticide exposures result in clinically overt poisoning. High pesticide exposure events, as a result of mishandling or equipment malfunction, can occur when mixing, loading and applying pesticides and during the repair and maintenance of pesticide application equipment. Fourteen percent of the pesticide applicators enrolled in the Agricultural Health Study (AHS) reported during their working lifetime at least one *high pesticide exposure event* (HPEE) (defined as "an incident or experience while using any pesticide which caused an unusually high personal exposure") [66]. The authors also reported that the majority of HPEEs did not result in a pesticide-associated health care visit. Another study of AHS participants found that only 50% of private pesticide applicators with a recent HPEE reported experiencing any symptoms related to the event [67].

HPEEs may be toxicologically important but are relatively understudied among agricultural workers. In particular, little is known about the neurotoxicity of unusually high pesticide exposures that do not result in overt poisoning. To address this question, we examined the associations between high pesticide exposure events and measures of central nervous system (CNS) function in private pesticide applicators in the Agricultural Health Study (AHS).

Materials and Methods

We conducted an epidemiological study of the association between NB function and HPEEs among participants enrolled in the AHS, a large, prospective study of licensed pesticide applicators from Iowa and North Carolina [50]. In 1993-1997, 52,394 private applicators enrolled in the AHS by completing a self-administered enrollment questionnaire at the time of pesticide licensing and recertification. A "take-home" questionnaire completed within one month of enrollment and two, five-year follow-up phone interviews have been administered to AHS participants. The most recent questionnaire was administered within a year of participation in this study. Information was collected on demographic characteristics, pesticide exposure, pesticide application methods, use of personal protective equipment, occupational exposure to other toxicants, and other activities that may influence exposure or disease risk. Copies of AHS questionnaires are available online [51].

Study Participants

Private pesticide applicators who completed all of the AHS questionnaires, resided in Iowa or North Carolina, and who lived within 150 miles of the testing facilities were invited to participate in the present study; an equal number of participants were selected from each state. AHS participants were not invited if they had previously

reported stroke, amyotrophic lateral sclerosis, multiple sclerosis, Parkinson's disease, retinal or macular degeneration, hypothyroidism or diabetes. In addition, participants who, during the most recent AHS interview, reported drinking more than 41 alcoholic beverages per week or reported being diagnosed with prior acute pesticide poisoning were also excluded. To study a population who were using pesticides agriculturally, the sample was limited to participants who were farming at the time of AHS enrollment. Women were also excluded because they represented fewer than 1% of licensed pesticide applicators in the AHS cohort. After the eligibility criteria were applied, 1,807 AHS participants were initially eligible to participate in the present study.

For the purposes of our study, a summary measure of lifetime days of use of all organophosphate (OP) pesticides reported at enrollment was created for each applicator so that individuals with higher lifetime use of OP pesticides could be oversampled. The 75th percentile of lifetime days of OPs was used as a cut point to create high and low OP exposure groups. Our goal was a study sample with equal numbers of participants from the top 25% of lifetime OP use category and from the remainder of the sample. Individuals in these groups were assigned a random number and were recruited in order from the lists. In Iowa, testing was conducted in Iowa City and Dubuque between November 2006 and March 2007. In North Carolina, testing was conducted in Greenville and Wilmington between January 2008 and March 2008. Participants were reimbursed for time and travel expenses. Appropriate Institutional Review Boards approved the study protocol and all participants provided written informed consent.

<u>High Pesticide Exposure Assessment</u>

Information on HPEEs was obtained from AHS interviews; each of the three surveys asked a slightly different question:

1. Have you ever had an incident or experience while using *any* type of pesticide which caused you unusually higher personal exposure?

2. Did you have any incidents with fertilizers, herbicides, or other pesticides that caused you an unusually high personal exposure?
3. Have you had any incidents or spills that resulted in an unusually high exposure to pesticides from contact with your skin, from breathing fumes or dust, or from accidental ingestion?

Participants who reported "yes" to at least one of the questions during an AHS interview were classified as having a HPEE. As we were interested in the effect of having at least one HPEE in a worker's lifetime, we combined responses from all three questionnaires to make an overall summary HPEE variable.

Neurobehavioral Testing

NB testing was administered to all participants by trained technicians unaware of the participants' exposure status. Eight computerized tests from the Neurobehavioral Evaluation System, Version 3 (NES3), [52-54] and the manual Grooved Pegboard test (Lafayette Instruments, Lafayette, IN) were administered. These tests have been used extensively in investigations of neurotoxicant-exposed persons and were selected to be sensitive indicators of a wide range of CNS functions. Briefly, the CNS functions assessed included sustained attention (Continuous Performance Test); visual scanning and processing speed (Digit-Symbol); motor speed (Finger Tapping test); fine motor coordination (Grooved Pegboard Test); verbal learning and memory (Hopkins Verbal Learning tests of Total Recall, Delayed Recall and Recognition); and motor speed and visual scanning (Sequences A and B tests).

Assessment of Potential Confounders

Potential confounding variables were considered *a priori* and were obtained from AHS data and the health history questionnaire administered on the day of NB testing. Information was obtained on age, height, education, state of residence, smoking status, alcohol and caffeine consumption, head injury, total lifetime days of pesticide use, use of personal protective equipment and exposure to other potential neurotoxicants such as solvents, and welding fumes. NES3 Adult Reading Test (ART) scores were measured to estimate intellectual functioning before the onset of injury or illness [58]. Positive and negative affectivity were measured using the NES3-administered version of the Positive and Negative Affect Schedule (PANAS) [59]. Visual acuity was measured using a standard testing instrument, the Optec 1000 (Stereo Optical Co, Chicago, IL). Possible visual acuity scores ranged from 20/20 to 20/200. Scores of 20/50 to 20/200 were considered indicators of poorer visual acuity.

Statistical Methods

Participants' data were excluded from the analysis of all NB outcomes for alcohol consumption on the day of testing, past diagnosis of alcoholism, reported drinking of $\geq$ 42 alcoholic beverages per week during the past year, brain tumor, macular degeneration, current use of anticonvulsant or psychiatric medication with known cognitive effects (e.g. benzodiazepines), and renal failure requiring dialysis. Results were excluded for one participant with severe dementia and for one participant who reported being struck by lightning. In addition, a small number of participants were excluded from individual tests after standard linear regression diagnostics were performed. Regression diagnostics included studentized residual plots and checks for leverage and influence [68]. Two subjects were excluded from Digit-Symbol, two from Continuous Performance Test, one from Sequences A and one from Sequences B models. These observations were found to be extreme outliers from the overall sample and each had a studentized residual value that

exceeded the absolute value of 4.0. All exclusion criteria were applied without reference to exposure information or NB testing results.

We created separate base models of outcome-specific covariates for each outcome measure using the following procedure. First, we examined the unadjusted association between each covariate and each outcome with linear regression. Covariates associated with a NB outcome with a p-value <0.20 were then selected for inclusion in an initial full multiple linear regression base model for that outcome. Covariates with p-values $\geq$0.20 were removed sequentially from the initial full base model. The final base model for each NB outcome included only those covariates with p-values <0.20.

Adjusted associations between each NB outcome and the HPEE variable were estimated with linear regression models in which the outcome was regressed on the HPEE variable while controlling for the base model covariates. We inverted the parameter estimates for the timed tests (Continuous Performance, Digit-Symbol, Grooved Pegboard, Sequences A and B) so that lower scores indicated poorer test performance for all NB outcomes.

To compare HPEE age-equivalent effect sizes across the neurobehavioral outcome measures, each adjusted HPEE parameter estimate was converted into an age-equivalent value by dividing it by the base model parameter estimate for age.

Sensitivity analyses. Individuals who reported a physician-diagnosed acute pesticide poisoning during the most recent AHS interview were excluded from the sample. However, we did not exclude participants who reported such poisoning during earlier AHS interviews. To evaluate whether associations between HPEEs and adverse NB outcomes were attributable to previous pesticide poisoning, we excluded participants who reported ever being diagnosed with pesticide poisoning from the analyses and the parameter estimates were compared to estimates from models that included these pesticide-poisoned individuals.

We used the P1RE1071201, P2RE1071202 and 07222008 releases of the AHS dataset and all analyses were performed using SAS software, version 9.2 (SAS Institute Inc., Cary, NC).

Results

Participation

NB testing was administered to 701 participants. Among 1,807 eligible participants, 42% agreed to participate and were scheduled for neurobehavioral testing. Of the 759 participants scheduled for testing, 58 participants either cancelled or failed to show for their scheduled appointment. The overall response rate was 39 percent.

Thirty-five (5%) participants were excluded from the statistical analyses because of medications (e.g. benzodiazepines), medical conditions (e.g., alcoholism), and other factors that may affect NB function. This resulted in 666 participants available for inclusion in the analyses. The 35 excluded individuals were similar to the 666 participants by state of residence, smoking history, weekly alcohol consumption, and cumulative lifetime exposure days to all pesticides (data not shown). Participants were however, slightly younger than those excluded from the analysis (61$\pm$12 years vs. 64$\pm$13 years) and were more likely to have completed greater than a high school education (50% vs. 40%) than those excluded from the analyses.

Characteristics of the Study Participants

Demographics. Among the 666 participants included in the analyses, 155 (23%) participants reported ever having experienced at least one HPEE. Of the participants with HPEEs, 54% were from Iowa and 46% were from North Carolina (Table 3.1). The use of personal protective equipment was reported in 87% of those with HPEEs and 86% of those

without HPEEs. In addition, eight individuals reported a previous diagnosis of acute pesticide poisoning; of these, seven participants also reported ever experiencing a HPEE.

Neurobehavioral outcome measures. Descriptive summary statistics for the NB test results are presented in Table 3.2. The Finger Tapping and Grooved Pegboard results were similar for both hands, therefore only the results of the dominant hand are presented. Some study participants were unable to complete the tests in the allowed time or after two attempts, or because of computer problems or test administrator error, consequently, the total number of participants completing each test varied. (Comparative values for neurobehavioral tests are presented in Appendix A).

Linear Regression Base Model Covariates

Base model regression coefficients for each NB outcome are presented in Table 3.3. For all outcome measures, age and Adult Reading Test (ART) scores were statistically significant covariates. State was included in the base models for all outcome measures with the exception of the Continuous Performance Test and HVLT Total Recall test. Visual acuity score was strongly associated with two tests which required visualization of small stimuli (Digit-Symbol and Grooved Pegboard). Lifetime days of pesticide use was a statistical significant covariate in HVLT Delayed Recall models. The total variance accounted for by the regression models was the highest for Digit-Symbol ($r^2 = 0.48$) and lowest for Finger Tapping ($r^2 = 0.17$).

Associations between High Pesticide Exposure Events and Neurobehavioral Outcomes

High pesticide exposure events were adversely associated with two of the nine neurobehavioral tests (Table 3.4). Participants with HPEEs were, on average, 4.9 seconds slower on the Digit-Symbol test than those without HPEEs. This effect size is equivalent to 4.6 years of age in this sample. Participants with HPEEs were, on average, 2.2 seconds

slower on the Sequences A test; an effect size equivalent to 3.4 years of age in this sample. No significant associations were observed for the seven other NB outcomes. The associations between HPEEs and test performance did not significantly differ by state. We saw no evidence of a positive association between HPEEs and the NB outcomes evaluated.

Sensitivity Analyses

To evaluate whether the results were related to previous pesticide poisoning, the eight participants who reported ever being diagnosed with pesticide poisoning were excluded and the analyses were rerun. The parameter estimate for Digit-Symbol was attenuated from 4.9 to 4.6 seconds and remained statistically significant. All other associations were unaffected by the exclusion of participants with previous pesticide poisoning.

Discussion

High pesticide exposure events are a relatively common event among farmers, with 20% reporting at least one such event in their lifetime. We observed modest but meaningful associations between HPEEs and adverse test performance on two of nine NB tests. The most sensitive test employed in this study was the Digit-Symbol test. The Digit-Symbol test is one of the most widely used and sensitive tests in neurotoxicology research [11]. Our results suggest that high-level pesticide exposures that do not result in overt poisonings may contribute to persistent adverse effects on visual scanning and processing among pesticide applicators.

Previous investigations of the NB toxicity of pesticide exposure have primarily focused on four areas of research: 1) the acute toxicity of pesticide poisoning; 2) the long-term toxicity of pesticide poisoning; 3) the long-term toxicity of low-level pesticide exposure with previous pesticide poisoning; and 4) the long-term toxicity of low-level

pesticide exposure without previous pesticide poisoning. Regarding the first three areas of research, there is a general consensus that acute pesticide poisoning is associated with central nervous system impairment. However, there is much debate whether prolonged low-level pesticide exposure, without evidence of previous poisoning, results in NB impairment. Several studies have reported a broad range of NB deficits in measures of memory, motor speed, simple reaction time, sustained attention and visual scanning and processing [24-25, 29, 46-47], while other studies have reported limited or no evidence of long-term neurobehavioral deficits [22, 26, 35, 69]. To our knowledge, this is the first study to examine the association between unusually high pesticide exposures and NB function among agricultural workers. Consequently, the results of this study fill a gap in knowledge about the long-term NB effects of pesticide exposure levels between low-level exposure and overt high level exposure resulting in pesticide poisoning.

Most studies have examined associations between neurological function and organophosphate pesticides, which are known human neurotoxicants. However, many other pesticides have neurotoxic properties with varying levels of toxicity [70-72]. For example, among the pesticide classes, insecticides generally have the most acute neurotoxicity, whereas herbicides generally have low to moderate acute neurotoxicity [1]. In the present study, we examined HPEEs which may have involved any pesticide. The dose of one pesticide necessary for a particular adverse effect may differ from the dose of another pesticide necessary for the same adverse effect. Such variability in exposure is likely to result in non-differential error and an attenuation of the observed exposure-effect associations. Furthermore, pesticides used in agricultural settings are the pesticide product which includes the pesticide active ingredient as well as other ingredients. These other ingredients can represent up to 99% of the product and may increase the toxicity of the pesticide [73-74]. As such, these chemical exposures may have contributed to the observed associations between HPEEs and measures of NB function. In addition, the definition of HPEEs was not consistent throughout the three surveys. We obtained self-

reported information on HPEEs from three AHS interviews using three slightly different questions. Although the questions about HPEEs varied slightly across the three AHS interviews, we can only assume these questions capture the same information.

The response rate to this study was low (39%). A low response rate suggests that our study sample may not have been fully representative of all eligible pesticide applicators enrolled in the AHS. The prevalence of HPEEs was higher in participants than in the non-participants (23% vs. 15%). However, there is no reason to believe that the exposure-effect association among participants is meaningfully different than the exposure-effect association among those who were eligible who did not participate. On several important characteristics, including age and total lifetime days of pesticide use, participants were similar to eligible non-participants, suggesting comparability between them.

A major strength of the study is that it was based on a relatively large sample of pesticide applicators randomly selected from the AHS, a population well characterized for lifetime pesticide use. The sample included pesticide applicators from two distinct geographic regions, in Iowa and North Carolina, with varying crops and farming practices. HPEEs were assessed at all three AHS interviews which preceded assessment of the NB outcomes. The outcomes used in this study were objective and unlikely to be influenced by exposure history. As such, the results of the present study are relevant to a large segment of the farming population.

It is unlikely that the observed adverse associations were the result of an acute cholinergic response to recent pesticide exposure or a previous acute pesticide poisoning event. First, a majority of people with a history of pesticide poisoning were excluded. Furthermore, when the eight study participants who reported pesticide poisoning at the time of AHS enrollment were excluded the results remained unchanged. Second, NB testing was conducted during the winter months when pesticide application is minimal. Additionally, given that signs and symptoms of clinically overt pesticide poisoning are

easily recognizable, we consider it unlikely that individuals with past pesticide poisoning failed to report these events.

In this sample of licensed pesticide applicators, a history of high pesticide exposure events was associated with adverse results on two NB tests. These findings add to the increasing evidence that pesticide exposure at levels that do not produce acute intoxication may be associated with long-term adverse neurological function. If these events do contribute to adverse neurological outcomes, then efforts aimed at preventing high pesticide exposures should be a public health priority.

Table 3.1. Demographic characteristics, personal health information, and chemical and pesticide exposures by high pesticide exposure events (HPEEs) among 666 pesticide applicators

Characteristic	No HPEEs (n=511)				HPEEs (n=155)			
	Mean	SD	No.	%	Mean	SD	No.	%
Age (yrs)	61.9	11.5	--	--	58.3	11.5	--	--
Adult Reading Test (0-60)	29.1	10.0	--	--	32.9	10.4	--	--
Positive affect (1-5)	3.6	0.7	--	--	3.5	0.6	--	--
Negative affect (1-5)	1.4	0.4	--	--	1.4	0.4	--	--
Lifetime pesticide use (days)	1,478	1,529			2,123	1,889		
Testing location								
Iowa	--	--	256	50	--	--	84	54
North Carolina	--	--	255	50	--	--	71	46
Education								
$\leq$ High school	--	--	274	54	--	--	60	39
> High school	--	--	237	46	--	--	95	61
Alcohol (drinks/wk)								
0 drinks	--	--	293	57	--	--	87	56
1-7 drinks	--	--	174	34	--	--	49	32
>7 drinks	--	--	44	9	--	--	19	12
Visual acuity								
20/20 - 20/40	--	--	428	84	--	--	138	89
20/50 - 20/200	--	--	83	16	--	--	17	11
Head injury								
No injury	--	--	403	79	--	--	107	69
Injury, no loss of consciousness	--	--	51	10	--	--	18	12
Injury, w/loss of consciousness	--	--	57	11	--	--	30	19
Caffeine use (drink regularly)	--	--	381	75	--	--	118	76
Solvents exposure (ever)	--	--	218	43	--	--	61	39
Personal protective equipment use			443	87			133	86
Physician diagnosed pesticide poisoning	--	--	1	<1	--	--	7	5

Table 3.2. Frequencies and means of neurobehavioral outcome measures by high pesticide exposure events (HPEEs) (n=666)

Outcome	No HPEEs (n=511)				HPEEs (n=155)			
	N	Mean	SD	Range	N	Mean	SD	Range
Continuous Performance (ms)	505	428.3	44.7	318.6 - 612.3	152	420.5	40.3	338.9 - 595.4
Digit-Symbol (s)	506	117.7	22.9	75.3 - 210.4	152	115.8	22.6	73.6 - 213.6
Finger Tapping, dominant hand (# of taps)	507	63.4	9.8	9 - 86	155	54.6	9.0	9 - 73
Grooved Pegboard, dominant hand (s)	511	92.7	24.2	51 - 180	154	86.9	20.6	57 - 159
HVLT Total Recall (# correct)	507	19.6	4.9	6 - 31	155	21.2	5.2	7 - 34
HVLT Delayed Recall (# correct)	507	6.5	2.8	0 - 12	155	7.2	2.7	0 - 12
HVLT Recognition (tp-fp)	507	8.3	2.6	-3 - 12	155	8.5	2.5	-3 - 12
Sequences A latency (s)	500	43.3	14.9	20.0 - 93.8	150	41.2	13.6	14.8 - 91.3
Sequences B latency (s)	491	65.4	21.7	29.7 - 144.4	149	60.1	18.0	22.8 - 114.7

NOTE: HVLT = Hopkin's Verbal Learning Test; tp = true positives; fp = false positives

Table 3.3. Base model regression coefficients for neurobehavioral outcome measures (n=666)

Outcome	Age (yrs)	ART score	Lifetime pesticide days	NA score	PA score	Caffeine	Education	State	Visual acuity score	Base model r^2
CPT[c] (ms)	-1.53[b]	0.60[b]	--	--	3.25[b]	-6.52	--	--	--	0.22
Digit-Symbol[c] (s)	-1.07[b]	0.43[b]	--	--	4.56[b]	--	3.03[a]	-4.92[b]	-4.79[a]	0.48
Finger Tapping, dominant (# of taps)	-0.23[b]	0.14[b]	--	--	0.97	--	--	-1.88[a]	--	0.17
Grooved Pegboard, dominant [c] (s)	-1.03[b]	0.15[b]	--	--	--	-3.26	--	-4.10[b]	-6.83[b]	0.34
HVLT Total Recall (# correct)	-0.18[b]	0.11[b]	--	-0.89[a]	0.67[b]	--	0.79	--	--	0.28
HVLT Delayed Recall (# correct)	-0.09[b]	0.05[b]	0.54[b]	-0.47[a]	0.37[a]	--	0.67[b]	-0.54[b]	--	0.27
HVLT Recognition (tp-fp)	-0.05[b]	0.05[b]	--	--	--	--	0.69[b]	-0.83[b]	--	0.20
Sequences A[c] (s)	-0.64[b]	0.39[b]	--	--	2.16[b]	--	--	-2.74[b]	--	0.41
Sequences B[c] (s)	-0.93[b]	0.53[b]	--	--	4.00[b]	--	--	-4.95[b]	--	0.42

NOTE: ART = Adult reading test; NA = negative affect; PA = positive affect; CPT = Continuous Performance Test; HVLT = Hopkin's Verbal Learning Test; tp= true positives; fp = false positives. Age, ART, Lifetime pesticide days (log_{10}), NA and PA are continuous variables. Caffeine, education, state of residence, and visual acuity are categorical variables. The reference groups for the categorical variables are: caffeine = drink regularly, education = $\leq$ high school education, state = Iowa, visual acuity = 20/20 - 20/40 vision

[a] $p<0.05$; [b] $p<0.01$; [c] Scores have been inverted so that lower scores indicate poorer performance.

Table 3.4. Adjusted linear regression models of associations between high pesticide exposure events (HPEEs) and neurobehavioral outcome measures

Outcome	N	β	95% CI	Age equivalence in years
CPT [a] (ms)	657	-0.17	-7.40, 7.05	0.1
Digit-Symbol [a] (s)	658	-4.91	-7.98, -1.83	4.6
Finger Tapping, dominant (# of taps)	662	-0.31	-1.94, 1.31	1.4
Grooved Pegboard, dominant [a] (s)	665	0.43	-3.11, 3.98	-0.4
HVLT Total Recall (# correct)	662	0.57	-0.23, 1.36	-3.2
HVLT Delayed Recall (# correct)	662	0.04	-0.41, 0.49	-0.4
HVLT Recognition (tp-fp)	662	-0.35	-0.77, 0.80	7.0
Sequences A[a] (s)	650	-2.24	-4.36, -0.13	3.5
Sequences B [a] (s)	640	-0.28	-3.31, 2.75	0.3

NOTE: CPT = Continuous Performance Test; HVLT = Hopkins Verbal Learning Test; tp= true positives; fp = false positives; Models are adjusted for the base model covariates listed in Table 3.3

[a] Scores have been inverted so that lower scores indicates poorer performance.

CHAPTER IV

PERIPHERAL NERVOUS SYSTEM OUTCOMES AMONG PESTICIDE APPLICATORS

Abstract

In the absence of acute pesticide poisoning, evidence that long-term exposure to organophosphate (OP) pesticides is associated with adverse peripheral nervous system (PNS) function among humans is limited. To investigate whether occupational exposure to OP pesticides is associated with impaired PNS function, we administered neurological tests to licensed pesticide applicators enrolled in the Agricultural Health Study (AHS). In 2006-2008, 678 male participants (mean age = 61 $\pm$ 12 years) completed a neurological physical examination (NPx), electrophysiological studies of the peroneal motor nerve, and tests of hand strength, sway speed and vibrotactile threshold. Information on lifetime use of 16 OP pesticides was obtained from AHS interviews in 1993-2007 and from a questionnaire administered at the time of neurological testing. Associations between pesticide use and measures of PNS function were estimated with linear and logistic regression while controlling for age and outcome-specific covariates. Five of six NPx outcomes (ankle reflex, postural tremor, tandem gait, toe proprioception and toe vibration) had at least one statistically significant adverse association with ever-use of one or more pesticides. Most notably, abnormal toe proprioception was significantly associated with ever-use of chlorpyrifos, coumaphos, dichlorvos, fonofos, phosmet, and tetrachlorvinphos with odds ratios ranging from 2.03 to 3.06; a monotonic increase in risk was observed for chlorpyrifos, fonofos, and phosmet. Mostly null associations were observed between OP pesticide use and electrophysiological tests, hand strength, sway speed and vibrotactile threshold. The results were unaffected by exclusion of eight

participants with past acute pesticide poisoning. In conclusion, this study provides some evidence that long-term occupational exposure to OP pesticides is associated with impaired PNS function in older male licensed pesticide applicators.

Introduction

Organophosphate (OP) pesticides are cholinesterase inhibiting agents widely used throughout the world to protect crops from insect damage. The EPA reported that, in 2001, 73 million pounds of OP pesticides were used in the United States accounting for approximately 70 percent of total US insecticide use [2]. Given the widespread use of these chemicals, exposure is common among agricultural workers and the potential for adverse health outcomes is considerable.

Acute, high-level exposure to OP pesticides can result in three distinct forms of neurotoxicity in humans – acute cholinergic toxicity (i.e., OP pesticide poisoning), the "intermediate syndrome", and an organophosphate-induced delayed polyneuropathy (OPIDP). The cholinergic syndrome is the result of inhibition of the enzyme acetylcholinesterase with subsequent overstimulation of cholinergic receptors. Symptoms of mild acute cholinergic toxicity include headache, dizziness, nausea, vomiting and diarrhea whereas more severe acute cholinergic toxicity includes cardiac rhythm disturbances, seizures, respiratory failure and coma. Although acute symptoms often resolve shortly after cessation of exposure, OP pesticide poisoning has been associated with long-term neurological sequelae, including deficits in impaired nerve conduction and increased prevalence of neurological symptoms [17, 19, 21, 35, 65]. The intermediate syndrome has also been described as a complication of acute exposure to OP pesticides. This syndrome develops in approximately 20 percent of individuals with cholinergic syndrome and is characterized by proximal limb muscle weakness and cranial nerve palsies [75]. Exposure to some OP pesticides (e.g. chlorpyrifos), that inhibit the

enzyme *neuropathy target esterase (NTE),* results in OPIDP, a rare condition which occurs several weeks following recovery from acute, high-level exposure [76-78]. Symptoms include muscle weakness, paralysis, pain, and paresthesia in a stocking-glove distribution and in more severe cases, impairment can be permanent.

Although the neurotoxicity of acute, high-level exposure to OP pesticides is well established, the consequences of long-term exposure at levels insufficient to cause clinical toxicity are more controversial. Research in this area has focused primarily on the neurobehavioral effects of long-term OP pesticide exposure. Most studies of non-poisoned individuals have found increases in self-reported neurologic symptoms and/or deficits in neurobehavioral function [24, 26, 28, 30, 46-47], reflecting central nervous system (CNS) impairment. However, few studies have examined the long-term toxicity of OP pesticides on the peripheral nervous system (PNS) in the absence of pesticide poisoning, and existing studies have produced mostly inconsistent results [26, 33-34, 36-38, 40, 79]. Furthermore, the literature is limited mostly to workers exposed to chlorpyrifos [26, 38, 40, 79]. The inconsistencies in previous studies are potentially due to imprecise estimates of pesticide exposure, variability in ascertainment of peripheral nervous system function and other methodological limitations such as small samples with fewer than 100 exposed individuals [31, 34, 37-39] and poor control of confounding by other neurotoxic agents (e.g. organic solvents).

The purpose of this investigation was to examine associations between estimates of cumulative lifetime exposure to specific OP pesticides and measures of peripheral nervous system (PNS) function in a large sample of pesticide applicators with well characterized lifetime exposure to OP pesticides. The primary hypothesis was that long-term OP pesticide use is associated with adverse peripheral neurological outcomes.

Materials and Methods

The AHS is a prospective study of over 89,000 private and commercial pesticide applicators and spouses of these applicators from Iowa and North Carolina [50]. In 1993-1997, 52,394 private pesticide applicators enrolled in the AHS at the time of pesticide licensing and recertification by completing a self-administered "enrollment" questionnaire. AHS participants were also administered a "take-home" questionnaire completed within one month of enrollment and two, five-year follow-up phone interviews; the most recent questionnaire was administered within a year of participation in the present study. Information was collected on pesticide use and application methods, use of personal protective equipment, demographic characteristics, exposures to other neurotoxicants (such as organic solvents), and other activities that may influence exposure or disease risk. Copies of AHS questionnaires are available online [51].

Study Participants

Private pesticide applicators were invited to participate in the present study who completed all of the AHS interviews, resided in Iowa or North Carolina, and lived within approximately 150 miles of the testing facilities. AHS participants who had previously reported a history of amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), Parkinson's disease (PD), stroke, retinal or macular degeneration, diabetes requiring medication and hypothyroidism were not invited to participate in the present study. In addition, participants who, during the most recent AHS interview, reported drinking more than 41 alcoholic beverages per week or reported being diagnosed with prior acute pesticide poisoning were also excluded. In order to study a population who were using pesticides agriculturally, the sample was limited to participants who were farming at the time of AHS enrollment. We also excluded women because they represented less than 1% of licensed pesticide applicators in the AHS cohort. A total of 1,807 AHS

participants were initially eligible to participate in the present study after eligibility criteria were applied.

We oversampled individuals with higher lifetime use of OP pesticides, by first creating a summary measure of lifetime days of use of all OPs for each applicator. We used the 75th percentile of lifetime days of use of all OPs as a cut point to create high and low OP exposure groups. Individuals in these two groups were assigned a random number and were recruited in order from the lists. Our goal was to recruit a study sample with equal numbers of participants from the high and low exposure groups. In Iowa, testing was conducted in two cities (Iowa City and Dubuque) between November 2006 and March 2007. In North Carolina, testing was also conducted in two cities (Greenville and Wilmington) between January 2008 and March 2008. Participants were reimbursed for time and travel expenses to and from the testing facility. Appropriate Institutional Review Boards approved the study protocol and all participants provided written informed consent.

Exposure Assessment

Pesticide use was quantified for each participant using information from the AHS questionnaires and a questionnaire administered at the time of neurological testing. Participants completed all phases of the AHS interview (enrollment, Phase 2, Phase 3). At enrollment, participants provided detailed information regarding lifetime use of 50 commonly used pesticides, including 10 OP and four carbamate insecticides. Information collected included ever-use, days/year used and number of years applied. The product of days/year and number of years was used to create lifetime days of use. Additionally at enrollment, participants completed a checklist of other chemicals; six from this list were OP pesticides that were used by at least 50 people. No information on frequency or duration of use was applied for these chemicals, so we assigned use history based on the average number of days per year that the individual applied insecticides, and

assumed that the individual had used that chemical since it came on the market, since they were 18 years of age, or the maximum years applied pesticides (whichever was lowest). The product of these values was used to create lifetime days for these chemicals. At the follow-up interviews (Phases 2 and 3), individuals reported the chemicals used in the past year (Phase 2) or since the last interview (Phase 3) and the number of days of use of that chemical. The questionnaire administered on the day of neurological testing provided pesticide use information for the past 12 months including ever mix or apply and duration of use (days). For these follow-up phases, the number of days used since last interview was calculated based on the number of years since the last interview. These days were summed with the data from enrollment to create lifetime days of use for each specific chemical. For OP use metrics, we used information on 15 OP pesticides reported on the AHS enrollment questionnaires (acephate, chlorpyrifos, coumaphos, diazinon, ethoprop, dichlorvos, dimethoate, disulfoton, fonofos, malathion, parathion, phorate, phosmet, terbufos, tetrachlorviphos) and, for a new chemical obtained during the two follow-up phone interviews (tebupirimfos). Tebupirimfos was introduced in 1995, therefore lifetime days was accumulated from the Phase 2 interview (1999-2003) forward. For individuals who reported using tebupirimfos on the Phase 2 interview, we assumed that their first year of use was in 1996. We excluded OP pesticides that were used by fewer than 50 people, resulting in detailed OP use information for 16 chemicals. A dichotomized pesticide exposure variable, *ever-use*, was also created for each pesticide for each participant. In addition to ever-use of specific OP pesticides and lifetime days of use of specific OP pesticides, a summary variable of OP pesticide use (cumulative lifetime days of all OP pesticides) was created for each participant.

We also obtained information on lifetime days of use of four carbamate pesticides (aldicarb, benomyl, carbaryl and carbofuran) from AHS questionnaires. Two exposure metrics were created for each of these carbamate pesticides from this information: ever-use and cumulative lifetime days of use.

From pesticide use information collected on AHS questionnaires and the questionnaire administered on the day of neurological testing, a cumulative lifetime days variable of all pesticide use variable was created. This variable included the 50 commonly used pesticides (both OPs and non-OPs) reported on the AHS enrollment questionnaire.

Information on high pesticide exposure events (HPEEs) was obtained from three AHS interviews; participants who reported "yes" to at least one of the following questions during an AHS interview were classified as having a HPEE:

1. Have you ever had an incident or experience while using *any* type of pesticide which caused you unusually higher personal exposure?
2. Did you have any incidents with fertilizers, herbicides, or other pesticides that caused you an unusually high personal exposure?
3. Have you had any incidents or spills that resulted in an unusually high exposure to pesticides from contact with your skin, from breathing fumes or dust, or from accidental ingestion?

We were interested in examining the association between having at least one HPEE in a worker's lifetime and PNS function; therefore, we combined responses from all three questionnaires to make an overall summary HPEE variable.

In summary, for every participant, pesticide exposures were characterized as: 1) ever-use of 16 OP and four carbamate pesticides; 2) cumulative lifetime days of use for each of these pesticides; 3) cumulative lifetime days of *all* OP pesticide use; 4) cumulative lifetime days of *all* pesticide use; and 5) ever having a high pesticide exposure event.

Neurological Outcome Measures

Neurological physical examination. A standard neurological physical examination was performed on all study participants by an experienced physician board-

certified in Internal Medicine and Occupational Medicine (FG) who was blinded to pesticide exposure status. The examination included assessment of sensory function, deep tendon reflexes, and movement and coordination. The sensory modalities, vibration and proprioception, were tested on the great toes, bilaterally. Toe vibration was assessed using a standard 128-Hz tuning fork. Proprioception was assessed by grasping the sides of the great toe and moving it in either flexion or extension until the participant indicated the direction of motion. Achilles deep tendon reflexes were examined bilaterally. The Romberg test was used to assess the ability to maintain upright posture without support (i.e. balance) and was performed with both eyes open and eyes closed. Tandem gait was examined to assess balance.

Clinical examination results were recorded as normal, equivocal, or abnormal. For postural tremor, Romberg and tandem gait, equivocal and abnormal results were collapsed into a single "abnormal" category so that, for each test, results were classified as either normal or abnormal. For ankle reflex, toe proprioception and toe vibration perception (i.e., the tests performed bilaterally), the examination outcome was classified as "abnormal" if ratings were either abnormal bilaterally, abnormal unilaterally with an equivocal finding on the contralateral side, or abnormal unilaterally with a missing value (due to injury/amputation) on the contralateral side. Neurotoxic chemicals generally do not cause asymmetrical neuropathy, therefore we did not classify an outcome as "abnormal" if ratings were abnormal unilaterally with a normal finding on the contralateral side.

Electrophysiological measures. Electrophysiological measures of the peroneal motor nerve were performed on study participants by the same examiner following the neurological physical examination. Foot temperature was monitored continuously and maintained above 32°C with heat lamps. The temperature was recorded at the beginning and end of the electrophysiological testing. Distal motor amplitude (mV), distal and proximal motor latency (ms), and short F-wave latency (ms) were obtained and nerve

conduction velocity (m/s) was calculated from the results. All electrophysiological measurements were made with a factory calibrated TECA Sapphire electromyograph (TECA Corp., Pleasantville, New York) using standard noninvasive techniques [80].

Hand strength. Hand strength dynamometry was performed bilaterally on study participants to assess neuromuscular function. Specifically, gross grip strength was obtained using a Grip Strength Dynamometer (JTech Medical, Salt Lake City, UT). Key pinch and palmar pinch measurements were obtained using a PinchTrack Dynamometer (JTech Medical, Salt Lake City, UT). Methods and instructions of Mathiowetz et al were employed [81]. For all hand strength dynamometry tests, three trials were administered for each hand with a short rest period between each trial. The average of the three trials (kg) was calculated for each hand. We calculated a z-score for each individual test and then created a mean z-score for all of the hand strength tests combined.

Sway speed. Sway speed, also known as standing or postural stability, was measured with a CATSYS 2000 Force Plate (Danish Product Development, Denmark) using a standard protocol [82]. The force plate was placed on a flat and level location on the floor approximately six feet from a wall. Participants were instructed to remove their shoes and stand erect on the platform with feet together. They were further instructed to look at a visual target on the wall and stand as still as possible during the testing. Four 80 second tests were administered, two with eyes open and two with eyes closed. Sway speed (mm/s) was recorded for each trial. Average sway speed was calculated for the two trials with eyes open and for the two trials with eyes closed.

Vibrotactile threshold. Cutaneous vibrotactile thresholds were obtained bilaterally for the great toe to assess acuity to vibration (an index of peripheral sensory function). The Vibratron II (Sensortek, Inc., Clifton, NJ) was used for measurements of vibrotactile threshold using the methods-of-limits protocol [32, 83-84]. Because no laterality was observed, a single mean vibrotactile threshold was calculated from the two

toe thresholds for each study participant. Vibrotactile thresholds are reported in log microns peak-to-peak amplitude.

Assessment of Potential Confounders

We considered potential confounding variables *a priori;* information on confounders was obtained from AHS data and the health history questionnaire administered on the day of neurological testing. Information on age, height, and smoking status was obtained from AHS interviews; education, state of residence, alcohol consumption, recent ear infection, history of inner ear surgery and exposure to other potentially neurotoxic substances such as solvents, soldering and welding fumes was obtained from the health history questionnaire. BMI (kg/m^2) was calculated using height information from the most recent AHS questionnaire (2006) and weight measurements obtained on the day of neurological testing.

Statistical Methods

Exclusions. Participants' data were excluded from the analysis of all neurological outcomes for alcohol consumption on the day of testing (n=3), past diagnosis of alcoholism (n=3), reporting drinking $\geq$ 42 alcoholic beverages per week during the past year (n=3), chemotherapy (n=4), diabetes (n=1), polio (n=6), and renal failure requiring dialysis (n=1). We also excluded the results for one participant with severe dementia and for one participant who reported being struck by lightning. Results were excluded for all tests except for the electrophysiological tests for five participants with a reported history of brain tumor. For tests of postural stability (Romberg, tandem gait and sway speed), we excluded two participants who reported currently using the drug meclizine (used to treat motion sickness) and two participants with Meniere's Disease (a disorder of the inner ear that can affect balance). Two participants were excluded from the nerve conduction velocity measure after standard linear regression diagnostics were performed. These

observations were found to be extreme outliers from the overall sample and each had a studentized residual value that exceeded the absolute value of 6.0. In addition, a small number of participants were unable to perform certain neurological tests due to recent surgery, limb amputation or injury. All exclusion criteria were applied without reference to exposure information or neurological testing results.

Logistic regression analyses. Logistic regression was used to evaluate associations between pesticide use and dichotomized (normal/abnormal) neurological physical examination results. A base model was developed using goodness-of-fit tests. Adjusted models were run with each individual pesticide use parameterized as ever vs. never-use with never-users as the referent group. Dose-response was also examined by creating a three-level variable for individual pesticides with the distribution of lifetime days of use split at the median among the pesticide users to create two exposure categories (median or less, and greater than the median) with never-use as the referent category. For the pesticide summary variables (lifetime days of all OP pesticides and lifetime days of all pesticides), the distribution was split in quartiles with the lowest exposure category as the referent group. Chi-square tests for trend were performed for individual pesticides (across the three categories) and for the pesticide summary variables (across the four categories). Analyses were restricted to pesticides with at least five exposed cases.

Linear regression analyses. Linear regression was used to examine associations between pesticide use and continuous peripheral neurological test results. We first created a base model for each neurological outcome measure with an outcome-specific set of covariates. Unadjusted linear regression analyses were performed to examine the association between each covariate and each outcome measure. Covariates associated with a neurological outcome with a p-value <0.20 were selected for inclusion in an initial full multiple linear regression base model for that outcome. Covariates with p-values $\geq$

0.20 were removed sequentially from the initial full base model. The final multivariate base model for each outcome included only those covariates with p-values <0.20.

The cumulative lifetime days of pesticide use variables were log transformed to normalize the distribution and meet linear regression assumptions. Each pesticide was examined both as a continuous variable (log_{10} lifetime days of use) and as a dichotomized variable (ever/never-use). Adjusted associations between neurological outcomes and pesticide exposures were estimated with linear regression models in which the neurological outcome was regressed on the pesticide exposure variable while controlling for the base model covariates. Parameter estimates for distal latency, short f-wave latency, sway speed (with both eyes open and closed) and vibrotactile threshold were inverted so that lower scores indicated poorer test performance for all neurological outcomes.

Interaction by state was examined with the inclusion of a state by pesticide interaction term. Standard linear regression diagnostics were performed on all models; regression diagnostics included studentized residual plots and checks for leverage and influence [60]. Extreme observations were identified and examined for plausibility.

Confounding by related pesticide exposures. Because pesticide applicators typically use more than one pesticide, we examined potential confounding of the association between neurological outcomes and each pesticide by other pesticides for both linear and logistic regression models. Specifically, Spearman correlations were calculated for pesticides associated with outcomes with a p- value <0.10. Moderately correlated pesticide pairs ($r \geq 0.30$) were added simultaneously to final base models. We then compared the pesticide variable parameter estimates to models with only one pesticide.

Sensitivity analyses. Eight individuals reported being diagnosed with acute pesticide poisoning during earlier AHS interviews. To evaluate whether any statistically significant associations between pesticide use and adverse neurological outcomes were

related to previous pesticide poisoning, the eight participants who reported ever being diagnosed with pesticide poisoning were excluded from the analyses. The results were then compared to the results from models that included these pesticide-poisoned individuals.

We used the P1RE1071201, P2RE1071202 and 07222008 releases of the AHS dataset and all analyses were performed using SAS software, version 9.2 (SAS Institute Inc., Cary, NC).

Results

Participation

Neurological testing was administered to 701 participants resulting in an overall response rate of 39%. Among 1,807 eligible participants, 953 (53%) refused to participate in the study when contacted by telephone and an additional 95 (5%) could not be reached after ten attempts. Forty-two percent of those eligible agreed to participate and were scheduled for testing. Of the 759 participants scheduled for testing, 58 cancelled or failed to show for their scheduled appointment. Twenty-three (3.4%) participants were excluded from all tests resulting in 678 participants available for inclusion in the analyses.

Characteristics of the Study Participants

Demographics. The mean age of the participants was 61.2 years (SD = 11.6) (Table 4.1). Approximately half of the participants reported completing at least a high school education; eight (1%) reported a previous diagnosis of acute pesticide poisoning.

Pesticide exposures. Information on pesticide use history is presented in Table 4.2. Most participants reported ever using any OP (98%) and all but one participant

reported lifetime use of at least one pesticide. OP use ranged from 77% for malathion to less than 10% for dimethoate, tebupirimfos and tetrachlorvinphos. Carbaryl (63%) was the most commonly used carbamate pesticide. OP pesticide use was similar between Iowa (98%) and North Carolina participants (97%) (data not shown). However, carbamate pesticide use was more common in North Carolina (93%) than in Iowa (67%).

Neurological outcome measures. Descriptive summary statistics for the neurological physical examination outcomes, electrophysiological tests and quantitative functional PNS tests (hand strength, sway speed and vibrotactile threshold) are presented in Table 4.3. Less than 10% of the study participants had abnormal Romberg and toe proprioception examination results, whereas 28% of the participants had abnormal tandem gait examination results. (Comparative values for neurological tests are presented in Appendix A).

Base Model Covariates

Base model covariates for the linear and logistic regression models are present in Table 4.4. Odds ratios (OR) are presented for the dichotomized neurological physical examination outcomes and parameter estimates are presented for the continuous electrophysiological and quantitative functional outcomes. For consistency across all continuous outcomes, distal latency, short F-wave and sway speed results were inverted so that negative parameter estimates indicated poorer performance. Age was a statistically significant covariate for all neurological outcome measures. Height was included in all models except for ankle reflex and postural tremor examination results. The total variance (r^2) accounted for by the linear regression models ranged from 0.16 for distal motor amplitude to 0.40 for hand grip strength.

Associations between Neurological Physical Examination Results and Pesticide Use

Overall, statistically significant adverse associations were observed for at least one of the six neurological physical examination outcomes and ever-use of 10 individual OP pesticides (Table 4.5.) The associations were strongest for toe proprioception and postural tremor; dose-response models for these outcomes are presented in Table 4.6. Inverse associations were observed for ever-use of three OP pesticides and all four carbamate pesticides. No significant associations were observed between the physical examination results and lifetime days of all OP pesticides or lifetime days of all pesticides (data not shown). Results for each neurological physical examination outcome are presented below.

Ankle reflex. Both, ever-use of phosmet and ever-use of tebupirimfos were associated with abnormal ankle reflex, while ever-use of aldicarb had an inverse association. Phosmet showed a dose response trend with an increase in OR across the two exposure groups ($OR_{\leq \text{median days}} = 2.81$, 95% confidence interval (CI): 1.19, 6.61; $OR_{> \text{median days}} = 2.93$, 95% CI: 1.29, 6.68 $p_{trend} < 0.05$). Due to small counts, we were unable to examine a dose-response for tebupirimfos. No evidence of a dose-response was found for aldicarb (data not shown).

Postural tremor. Adverse associations were observed for ever-use of dimethoate, disulfoton, ethoprop and tebupirimfos and postural tremor with ORs ranging from 1.90 to 2.17. A statistically significant test for trend was observed for lifetime days of dimethoate, disulfoton, ethoprop and tebupirimfos, though we did not observe a monotonic increase in risk with disulfoton. We also observed an inverse association for ever-users of diazinon and postural tremor, with no evidence of a dose-response relationship.

Romberg test. We observed an inverse association for ever-use of carbofuran. There was also some evidence of a dose-response for chlorpyrifos ($OR_{\leq \text{median}} = 1.29$,

95% CI: 0.65, 2.57; OR $_{>median}$ = 1.66, 95% CI: 0.79, 3.48; p_{trend} = 0.18). No other statistically significant associations were observed between the Romberg test and pesticide use.

Tandem gait. An adverse association was observed for ever-use of dichlorvos and tandem gait, whereas ever-use of acephate and benomyl were inversely associated. However, no evidence of a dose-response relationship with tandem gait was observed for dichlorvos, acephate or benomyl (data not shown). While the association between ever-use of phosmet and tandem gait was not significant, there was evidence of a dose-response relationship when the three use categories were analyzed ($OR_{\leq median}$ = 1.20, 95% CI: 0.50, 2.89; OR $_{>median}$ = 2.31, 95% CI: 1.07, 4.97 p_{trend} = 0.04).

Toe proprioception. Adverse associations were observed between toe proprioception and ever-use of chlorpyrifos, coumaphos, dichlorvos, fonofos, phosmet and tetrachlorvinphos with ORs ranging 2.03 to 3.06. An inverse association was observed among ever-users of the carbamate insecticide, carbaryl . In the dose-response models, chlorpyrifos, dichlorvos, fonofos, phorate, phosmet, and carbaryl all had significant tests for trends, though dichlorvos did not show a monotonic increase in risk. We were unable to examine the dose-response relationship between several pesticides and toe proprioception due to small counts.

Toe vibration. Adverse associations were observed between toe vibration and ever-use of dichlorvos (OR = 1.94, 95% CI: 1.32, 3.31) and ever-use of tetrachlorvinphos (OR = 2.15, 95% CI: 1.11, 4.17). There was evidence of a dose-response for dichlorvos ($OR_{\leq median}$ = 1.89, 95% CI: 0.95, 3.70; $OR_{>median}$ = 2.00, 95% CI: 1.00, 4.01; p_{trend} = 0.04). Counts were too small to examine a dose-response for tetrachlorvinphos.

Associations between Electrophysiological Measures and Pesticide Use

Adjusted associations between four electrophysiological measures of the peroneal motor nerve and pesticide use are presented in Table 4.7. The parameter estimate (β) for ever-use of each pesticide represents the difference in outcome mean between ever-users and never-users of that pesticide, adjusted for base model covariates. Results were inverted for distal motor latency and short F-wave measures so that negative parameter estimates indicated poorer test results for all tests.

Overall, most associations were in the positive direction, indicating better electrophysiological test results with pesticide use. Lifetime days of all OP use was not significantly associated with any of the electrophysiological measures. For phorate, and all pesticides combined, we observed a significant state by pesticide interaction. Individuals who reported ever experiencing a high pesticide exposure event had a significantly better result on one test compared to those without a high pesticide exposure event. Outcome specific results are presented below.

Distal motor amplitude. Both ever-use and lifetime days of use of tebupirimfos and phorate were significantly associated with better (greater) distal motor amplitude. The association between lifetime days of phorate use and distal motor amplitude differed by state (p-interaction = 0.04). Among Iowa participants, lifetime days of phorate was associated with better amplitude (β=0.49, 95% confidence interval (CI): 0.17, 0.80) whereas a null association was observed in North Carolina (β= -0.02, 95% CI: -0.41, 0.36). We also observed a significant association between HPEEs and better distal motor amplitude.

Distal motor latency. Diazinon ever-use and lifetime days of use were both significantly associated with longer (poorer) distal motor latency. Lifetime days of carbaryl use and lifetime days to all pesticides was also associated with adverse distal motor latency. The association between lifetime days and ever-use of phorate and distal

motor latency differed by state (p-interaction <0.01). Iowa participants who reported ever using phorate had better, but non-significant, distal motor latency (β= 0.14, 95% CI: -0.03, 0.31), while North Carolina participants had an adverse association (β= -0.21, 95% CI: -0.42, 0.00). Also, among Iowa participants, lifetime days of phorate was significantly associated with better distal motor latency (β= 0.10, 95% CI: 0.00, 0.20) whereas a nearly statistically significant adverse association was observed among North Carolina subjects (β= -0.11, 95% CI: -0.24, 0.01). Additionally, lifetime days of all pesticides was associated with poorer distal motor latency among North Carolina participants (β= -0.19, 95% CI: -0.35, -0.04) while no significant association was observed among Iowa participants (β= 0.08, 95% CI: -0.13, 0.29).

Nerve conduction velocity. Lifetime days of phorate use was significantly associated with better (faster) nerve conduction velocity.

Short F-wave latency. We observed significantly better (shorter) short F-wave latency for both ever-use and lifetime days of acephate, phorate and aldicarb use. In addition, lifetime days to all pesticides was significantly associated with better short F-wave latency. No significant adverse associations between short F-wave latency and pesticide use were observed.

Associations between Quantitative Functional Measures and Pesticide Use

The results of the multiple linear regression models used to estimate adjusted associations between quantitative functional measures and pesticide exposure variables are presented in Table 4.8. At least one positive association with ever-use or lifetime days of pesticide use was observed for each quantitative functional test. Ever experiencing HPEEs was associated with significantly better hand strength compared to individuals without HPEEs. No statistically significant negative associations were observed. Outcome specific results are presented below.

Hand strength z-score. Ever-use and lifetime days of use of phosmet were both significantly associated with greater hand strength. We also observed a significant state by pesticide interaction for ever-use of parathion and lifetime days of parathion use (p-interaction <0.01). Among Iowa participants, lower hand strength was associated with ever parathion use (β= -0.28, 95% CI: -0.48, -0.07) and lifetime days of parathion use (β= -0.19, 95% CI: -0.30, -0.07). Conversely, among North Carolina participants, greater hand strength was associated with ever parathion use (β= 0.23, 95% CI: 0.07, 0.39) and lifetime days of parathion use (β= 0.16, 95% CI: 0.05, 0.27). HPEEs were significantly associated with greater hand strength.

Sway speed. Ever-use and lifetime days of parathion, tebupirimfos and aldicarb were each significantly associated with better sway speed with eyes open. Lifetime days and ever-use of aldicarb were also associated with better sway speed with eyes closed.

Vibrotactile threshold. Ever-use and lifetime days of aldicarb use was associated with better vibrotactile threshold. We did not observe adverse associations between vibrotactile threshold and pesticide use and we did not find significant state by pesticide interactions.

Confounding by Correlated Pesticide Exposures

The addition of correlated pesticides to the single pesticide linear regression models did not attenuate any statistically significant associations between the pesticide exposures and the electrophysiological or quantitative functional outcome measures. However, some associations between the neurological physical examination tests and pesticides were modestly attenuated with the addition of correlated pesticides. For toe proprioception, the OR for ever-use dichlorvos was attenuated from 2.73 (95% CI: 1.53, 4.86) to 2.45 (95% CI: 1.32, 4.54) when ever-use of coumaphos was added to the model; the OR for coumaphos alone was attenuated from 2.03 (95% CI: 1.06, 3.90) to 1.42 (95% CI: 0.71, 2.92). For postural tremor, the OR for ever-use of ethoprop alone was

attenuated from 2.16 (95% CI: 1.35, 3.47) to 1.90 (95% CI: 1.05, 3.43) when ever-use of disulfoton was added to the model; the OR for disulfoton increased from 1.28 (95% CI: 0.69, 2.39) to 1.95 (95% CI: 1.19, 3.18).

Sensitivity Analyses

To evaluate whether the present results were driven by previous pesticide poisoning, the eight participants who reported past physician diagnosed pesticide poisoning were excluded from the sample and the analyses were rerun. When poisoned individuals were removed from the analysis, no estimate of association changed by more than ten percent and most showed essentially no change.

Discussion

Organophosphate pesticides are known neurotoxicants; however, relatively few studies have examined the association between OP pesticide use and PNS function among non-pesticide-poisoned individuals. In the present study, we identified significant adverse associations between use of several OP pesticides and neurological physical examination results. Dichlorvos use was most often associated with abnormal test results (i.e. tandem gait, toe proprioception and toe vibration). Toe proprioception was the most sensitive physical examination test and was adversely associated with six of 16 OP pesticides. Significant dose-response relationships were observed for most of these of the pesticides, suggesting that the associations were not spurious. Interestingly, we observed mostly null associations between OP pesticide use and other peripheral neurological outcomes, including electrophysiological measures, hand strength dynamometry, sway speed and vibrotactile threshold. Furthermore, we observed several improved measures of PNS function with the carbamate pesticides. Aldicarb, for example, was positively associated with five tests (ankle reflex, short f-wave latency, sway speed with eyes open

and eyes closed, and vibrotactile threshold). Despite the inconsistency in findings across the measures of peripheral nervous system function used in this study, our results provide some evidence that long-term exposure to specific OP pesticides may adversely affect the peripheral nervous system.

Other studies examining the neurological health effects of long-term OP pesticide exposure, in the absence of previous poisoning, have also reported inconsistent associations with measures of PNS function. For example, one study evaluated neurological signs and symptoms and vibration threshold in 123 OP pesticide applicators (mean age = 36 years) and 123 non-applicators (mean age = 37 years) [33]. After controlling for confounding, pesticide applicators had significantly elevated odds ratios for peripheral neuropathy symptoms, motor coordination signs, deep tendon reflexes and reduced muscle power compared to referents. In addition, a borderline association ($p=0.08$) was observed for vibrotactile threshold, while no association was observed for toe vibration abnormality on physical examination. Another study assessing neurological symptoms, vibrotactile thresholds and motor tremor among 164 OP pesticide applicators (mean age = 34 years) and 83 unexposed controls (mean age = 33 years) observed strong associations between neurological symptoms and exposure status [35]. However, no associations were observed for vibrotactile threshold or motor tremor. In a study of 191 OP termiticide applicators (mean age = 39 years), and 106 unexposed friends (mean age = 38 years) and 83 unexposed workers (mean age = 43 years), the OP-exposed termiticide applicators did not differ significantly from the referents on neurological clinical examination outcomes or on measures of vibrotactile sensitivity [26]. Mean sway speed (with eyes open), however, was significantly longer among the applicators than among the referents.

The studies described above all used a dichotomized exposure metric (e.g. pesticide applicators vs. non-pesticide applicators) based on job title or occupational classification. The use of such exposure metrics results in the mixing of a wide range of

actual exposures into a few categories. In the present study, we did not use an unexposed comparison population. Rather, we examined neurological function in a large sample of pesticide applicators with exposure ranging from essentially no exposure to more than 30 years of exposure. This study design reduced the risk of inappropriate comparisons and potential confounding. We also used relatively precise estimates of exposure. The Agricultural Health Study has prospectively collected detailed information on pesticide use since 1993. Methodological studies have shown that AHS participants provide accurate and reliable pesticide use and duration of pesticide exposure information [62-64]. We used existing AHS data to estimate lifetime exposure to 16 specific OP pesticides, as well as to this class as a whole for each participant. In addition, because carbamate pesticides share a similar pathophysiology with organophosphate pesticides (i.e. acetylcholinesterase inhibition), we also estimated lifetime exposure to four specific carbamate pesticides. Since we did not have complete information on the frequency or duration of use for a few pesticides, we assigned use history for these chemicals based on other pesticide information from AHS interviews. Therefore, it is possible there was non-differential exposure misclassification of these pesticides. This may have resulted in over or underestimation of the exposure-effect association depending on the magnitude of the misclassification.

It is unclear why we observed several significant adverse associations between pesticide use and the neurological physical examination tests but not for the analogous quantitative measures. For example, we observed significant adverse associations between toe vibration (a neurological physical examination test) and two OP pesticides (dichlorvos and tetrachlorvinphos). However, we did not observe an association between the quantitative vibrotactile threshold test and these chemicals. Other studies have also reported differences between physical examination results and analogous quantitative measures [26, 33]. In the present study, it is possible that greater non-differential (random) error occurred in the quantitative tests in comparison to the physical

examination outcomes. Another possibility is that the physical examination tests better capture clinically relevant peripheral nerve impairment.

We observed a few significant interactions between state and indices of pesticide exposure. Most notably, we observed interactions between state and phorate use for two electrophysiological tests (distal motor amplitude and distal motor latency). Iowa participants had better results on these two tests, whereas North Carolina participants had poorer results. The explanation for these interactions is unclear. However, the difference in findings observed between Iowa and North Carolina participants may be due to differences in pesticide use and application methods.

The current study had several methodological limitations. First, the study sample may not have been representative of all AHS participants since just under 40% of eligible AHS subjects participated. However, demographic data were available to permit comparisons between participants and non-participants. On several important characteristics, including age and total lifetime days of pesticide use, participants were similar to eligible non-participants, suggesting comparability between them. Furthermore, because eligibility for the current study required completion of all AHS questionnaires, those who were most adversely affected by pesticide exposure may not have met this criterion. This concern is amplified by the fact that participation in the current study required participants be sufficiently physically robust to travel to the study site and participate in several hours of evaluation. Thus, it is likely that the study sample was at least somewhat selected by loss of those most susceptible to the adverse effects of pesticide exposure.

Second, we presented the results of numerous statistical tests. No formal correction for these multiple comparisons was made during the data analysis. To reduce the number of comparisons, we combined six hand strength measures into a single summary hand strength z-score, and we combined the vibrotactile thresholds of both the dominant and non-dominant great toe. In addition, for several physical examination tests

that were administered bilaterally, we collapsed the results into one dichotomized outcome. However, given that we still performed hundreds of statistical tests and observed significant associations in both positive and negative directions, we cannot rule out the possibility that some of our findings may be due to chance. For models with ever-use of individual pesticides we performed 280 statistical tests and observed 16 (5.7%) adverse associations and 11 (3.9%) positive associations. By chance alone at a p=0.05 level of significance, we would have expected 14 statistically significant associations.

It is unlikely that the adverse associations observed in this study were the result of previous pesticide poisoning. We did not recruit AHS participants who reported a past diagnosis of pesticide poisoning at the most recent AHS interview. Furthermore, in a sensitivity analysis, we excluded the eight participants who reported a previously diagnosed pesticide poisoning at the time of initial AHS enrollment (but not the most recent AHS interview). The exclusion of these individuals did not affect the overall interpretation of our findings. It is also unlikely that current pesticide toxicity accounts for the observed associations since evaluations were performed during the non-pesticide application season. In addition, because pesticide applicators are potentially exposed to a number of other neurotoxic chemicals, such as organic solvents, soldering and welding, we screened these chemicals for their potential to confound. These variables were not significantly associated with any of the PNS outcome measures; therefore, we find it unlikely that exposure to these chemicals could explain the exposure-effect associations observed in this study. However, we cannot rule out the possibility of confounding by other unmeasured neurotoxic chemicals.

In summary, our findings indicate that long-term exposure to some OP pesticides is adversely associated with indices of PNS function among pesticide applicators with no previous history of pesticide poisoning. Most notably, abnormal toe proprioception was significantly associated with ever-use of chlorpyrifos, coumaphos, dichlorvos, fonofos, phosmet, and tetrachlorvinphos; a monotonic increase in risk were observed for

chlorpyrifos, fonofos, and phosmet. The adverse associations observed in this study were independent of previous pesticide poisoning and are likely due to long-term pesticide exposure.

Table 4.1. Demographic characteristics, personal health information and chemical exposure among licensed pesticide applicators (n=678)

Characteristic	Mean	SD	Min.	Max.	No.	%
Age (yrs)	61.2	11.6	31.7	94.3	--	--
Height (cm)	179.1	6.4	154.9	200.7	--	--
BMI (kg/m^2)	28.7	4.0	17.9	46.3	--	--
Foot temperature (°C)	31.9	0.8	29.5	34.7	--	--
Testing location						
Iowa	--	--	--	--	342	50.4
North Carolina	--	--	--	--	336	49.6
Education						
$\leq$ High school	--	--	--	--	344	50.7
> High school	--	--	--	--	334	49.3
Smoking status						
Never smoked	--	--	--	--	387	57.3
Current smoker	--	--	--	--	44	6.5
Past smoker	--	--	--	--	244	36.2
Alcohol consumption (drinks/wk)*						
0 drinks	--	--	--	--	390	57.5
1-7 drinks	--	--	--	--	245	36.1
>7 drinks	--	--	--	--	43	6.3
Pesticide poisoning	--	--	--	--	8	1.2
Solvent exposure	--	--	--	--	279	41.2
Soldering exposure	--	--	--	--	34	5.0
Welding exposure	--	--	--	--	135	19.9
Inner ear surgery	--	--	--	--	14	2.1
Ear infection in the past 12 months	--	--	--	--	17	2.5

* The average number of drinks per week during the past 12 months

Table 4.2. Frequencies and means of lifetime days of pesticide use among licensed pesticide applicators (n=678)

Pesticide exposure	N*	%	Mean	SD	Min.	Median	Max.
Organophosphates							
Acephate	163	24.0	86.2	90.9	2.5	56.0	500.5
Chlorpyrifos	406	59.9	68.0	101.7	2.0	27.8	767.3
Coumaphos	90	13.3	59.3	184.6	1.0	10.3	1,627.5
Diazinon	294	43.4	54.2	93.0	1.0	20.0	846.0
Dichlorvos	123	18.1	417.6	927.4	1.0	56.0	5,880.0
Dimethoate	64	9.4	47.1	69.2	2.0	24.5	457.3
Disulfoton	107	15.8	42.9	42.1	2.0	24.5	236.0
Ethoprop	119	17.6	45.5	49.8	2.5	24.5	316.0
Fonofos	195	28.8	63.5	82.4	2.0	38.8	457.3
Malathion	525	77.4	87.7	196.3	2.0	26.0	2,625.0
Parathion	143	21.1	102.9	274.6	1.0	20.0	1,667.5
Phorate	218	32.2	72.3	137.6	2.0	27.0	1,627.5
Phosmet	99	14.6	61.6	83.7	2.5	27.3	600.0
Tebupirimfos	64	9.4	50.7	47.0	4.0	39.5	250.0
Terbufos	344	50.7	90.8	104.4	2.0	56.0	752.3
Tetrachlorvinphos	66	9.7	66.7	100.9	3.0	24.5	581.6
Carbamates							
Aldicarb	127	18.7	82.2	114.6	2.0	26.0	600.5
Benomyl	112	16.5	62.6	123.7	1.0	15.4	767.3
Carbaryl	430	63.4	91.5	134.5	1.0	38.8	1,237.5
Carbofuran	281	41.5	54.8	91.4	1.0	24.5	752.3
Summary variables							
All organophosphates	661	97.5	395.4	612.4	2.0	224.8	5,959.3
All pesticides	677	99.9	1,619.5	1,634.4	10.0	1,045.5	11,676.8
HPEEs (ever)	158	23.3	--	--	--	--	--

NOTE: HPEEs = high pesticide exposure events

* Number of participants who reported ever-use.

Table 4.3. Descriptive statistics for neurological physical examination tests, electrophysiological tests and quantitative functional PNS tests among licensed pesticide applicators (n=678)

Outcome	N	Abnormal N	Abnormal %	Mean	SD	Min.	Max.
Neurological physical examinations							
Ankle reflex	663	109	16.4	--	--	--	--
Postural tremor	664	117	17.6	--	--	--	--
Romberg	645	59	9.2	--	--	--	--
Tandem gait	641	180	28.1	--	--	--	--
Toe proprioception	665	62	9.3	--	--	--	--
Toe vibration	664	120	18.1	--	--	--	--
Electrophysiological tests*							
Distal motor amplitude (mV)	665	--	--	4.9	2.6	0.0	13.9
Distal motor latency (ms)	656	--	--	5.1	0.9	3.3	9.3
Nerve conduction velocity (m/s)	653	--	--	44.1	4.4	25.0	56.8
Short F-wave latency (ms)	545	--	--	53.0	5.0	42.1	77.9
Quantitative functional PNS tests							
Hand strength (kg)							
Grip, d.	666	--	--	38.2	9.6	9.6	72.9
Grip, nd.	668	--	--	38.2	9.9	8.7	70.9
Key pinch, d.	665	--	--	10.9	2.0	1.3	16.5
Key pinch, nd.	667	--	--	10.8	2.1	2.7	19.7
Palmar pinch, d.	665	--	--	9.9	2.3	0.7	16.7
Palmar pinch, nd.	667	--	--	10.1	2.4	1.1	20.3
Sway speed - eyes open (mm/s)	655	--	--	14.6	5.1	5.0	38.6
Sway speed - eyes closed (mm/s)	656	--	--	22.9	10.2	6.4	70.6
Vibrotactile threshold, d. (log μ)	660	--	--	1.5	0.5	-0.4	2.4
Vibrotactile threshold, nd. (log μ)	661	--	--	1.5	0.5	-1.0	2.4

NOTE: d = dominant; nd = non-dominant; μ = microns

* Electrophysiological tests were performed on the peroneal motor nerve.

Table 4.4. Logistic and linear regression base model covariates for peripheral nervous system (PNS) outcome measures (n=678)

Outcome	N	Age (yrs)	BMI (kg/m^2)	Height (cm)	Foot temp. (°C)	State (N. Carolina)	Model r^2
Logistic regression results (odds ratios)							
Neurological physical examinations							
Ankle reflex	663	1.09[a]	1.11[a]	--	--	--	--
Postural tremor	664	1.04[a]	--	--	--	--	--
Romberg	645	1.12[a]	--	1.07[a]	--	0.43[a]	--
Tandem gait	641	1.11[a]	--	1.05[a]	--	--	--
Toe proprioception	665	1.03[a]	--	1.05[a]	--	--	--
Toe vibration	664	1.09[a]	--	1.13[a]	--	0.22[a]	--
Linear regression results (parameter estimates)							
Electrophysiological tests							
Distal motor amplitude (mV)	664	-0.08[b]	--	-0.08[b]	-0.33[b]	0.60[b]	0.16
Distal motor latency (ms) *	655	-0.01[a]	--	-0.02[a]	0.21[a]	1.06[a]	0.32
Nerve conduction velocity (m/s)	652	-0.15[b]	--	-0.21[b]	0.48[a]	-0.37	0.24
Short F-wave latency (ms) *	544	-0.15[a]	--	-0.40[a]	0.95[a]	0.12	0.36
Quantitative functional PNS tests							
Hand strength summary z-score	671	-0.03[b]	0.03[b]	0.02[b]	--	-0.54[b]	0.40
Sway speed - eyes open (mm/s) *	655	-0.24[a]	--	-0.19[a]	--	2.47[a]	0.33
Sway speed - eyes closed (mm/s) *	656	-0.40[a]	--	-0.42[a]	--	6.38[a]	0.31
Vibrotactile threshold summary (log μ) *	667	-0.02[b]	--	-0.02[b]	--	--	0.37

NOTE: Model r-square not reported for logistic regression models

[a] $p<0.05$; [b] $p<0.01$

* Scores have been inverted so that lower scores indicate poorer test results.

Table 4.5. Adjusted associations between neurological physical examination tests and ever-use of individual pesticides among licensed pesticide applicators (n=678)

Exposure	Ankle reflex (abnormal=109, normal = 554)		Postural tremor (abnormal = 117, normal = 547)		Romberg (abnormal = 59, normal = 586)		Tandem gait (abnormal = 180, normal = 586)		Toe proprioception (abnormal = 62, normal = 603)		Toe vibration (abnormal = 120, normal = 554)	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Organophosphates												
Acephate	0.67	0.39, 1.16	1.39	0.89, 2.19	0.84	0.34, 2.08	**0.60**	**0.37, 0.97**	--	--	0.54	0.25, 1.18
Chlorpyrifos	0.81	0.52, 1.26	1.03	0.68, 1.56	1.43	0.78, 2.62	1.21	0.81, 1.82	**2.35**	**1.28, 4.31**	1.45	0.91, 2.30
Coumaphos	1.40	0.75, 2.63	0.93	0.50, 1.74	1.12	0.50, 2.54	1.05	0.60, 1.89	**2.03**	**1.06, 3.90**	1.27	0.70, 2.31
Diazinon	1.24	0.80, 1.93	**0.63**	**0.41, 0.96**	0.69	0.38, 1.26	1.07	0.72, 1.58	0.67	0.39, 1.16	1.02	0.65, 1.62
Dichlorvos	1.35	0.78, 2.34	0.81	0.46, 1.40	1.06	0.50, 2.23	**2.29**	**1.41, 3.71**	**2.73**	**1.53, 4.86**	**1.94**	**1.32, 3.31**
Dimethoate	1.20	0.58, 2.48	**1.90**	**1.01, 3.54**	0.94	0.34, 2.59	1.26	0.66, 2.43	1.86	0.86, 4.00	1.66	0.83, 3.31
Disulfoton	0.95	0.53, 1.70	**1.95**	**1.19, 3.18**	0.84	0.33, 2.15	0.80	0.47, 1.35	0.39	0.15, 1.01	0.67	0.30, 1.50
Ethoprop	1.03	0.58, 1.80	**2.16**	**1.35, 3.47**	1.52	0.65, 3.58	0.98	0.59, 1.64	**0.36**	**0.14, 0.93**	1.08	0.52, 2.23
Fonofos	1.47	0.92, 2.34	1.07	0.69, 1.66	0.90	0.45, 1.81	1.18	0.77, 1.80	**3.06**	**1.79, 5.25**	1.62	0.98, 2.68
Malathion	1.37	0.78, 2.34	0.88	0.54, 1.42	0.73	0.37, 1.43	0.82	0.51, 1.31	1.05	0.55, 2.00	1.20	0.69, 2.09
Parathion	0.95	0.57, 1.60	0.99	0.61, 1.61	0.72	0.34, 1.50	0.73	0.46, 1.16	0.34	0.14, 0.81	1.30	0.75, 2.25
Phorate	0.68	0.42, 1.11	0.81	0.52, 1.26	0.86	0.45, 1.63	1.05	0.70, 1.59	1.67	0.97, 2.84	0.99	0.61, 1.60
Phosmet	**2.87**	**1.52, 5.44**	0.64	0.31, 1.29	1.18	0.46, 3.01	1.71	0.93, 3.12	**2.82**	**1.47, 5.42**	0.73	0.38, 1.41
Tebupirimfos	**2.01**	**1.00, 4.05**	**2.17**	**1.18, 4.00**	--	--	1.00	0.51, 1.95	1.84	0.85, 4.01	0.86	0.42, 1.76
Terbufos	0.95	0.61, 1.48	0.89	0.59, 1.34	0.79	0.42, 1.47	1.08	0.73, 1.59	1.25	0.73, 2.13	1.02	0.64, 1.62
Tetrachlorvinphos	1.13	0.53, 2.34	1.36	0.70, 2.62	1.68	0.67, 4.24	1.01	0.50, 2.02	**2.35**	**1.11, 4.98**	**2.15**	**1.11, 4.17**
Carbamates												
Aldicarb	**0.43**	**0.22, 0.86**	1.37	0.84, 2.26	0.46	0.16, 1.31	1.00	0.61, 1.65	0.43	0.18, 1.02	1.09	0.53, 2.26
Benomyl	0.65	0.35, 1.22	1.26	0.75, 2.11	0.87	0.35, 2.18	**0.52**	**0.30, 0.90**	0.40	0.15, 1.02	1.28	0.64, 2.59
Carbaryl	0.67	0.42, 1.06	1.39	0.89, 2.16	0.74	0.38, 1.45	0.77	0.51, 1.17	**0.45**	**0.26, 0.77**	1.25	0.75, 2.07
Carbofuran	0.77	0.50, 1.21	0.95	0.63, 1.42	**0.48**	**0.26, 0.90**	0.95	0.64, 1.41	1.45	0.85, 2.46	1.23	0.79, 1.93
HPEEs	0.96	0.56, 1.67	1.29	0.80, 2.08	1.57	0.78, 3.15	1.03	0.63, 1.68	0.99	0.51, 1.90	0.68	0.38, 1.21

NOTE: OR = odds ratio; 95% CI = 95% confidence interval; HPEEs = high pesticide exposure events; Ankle reflex models were adjusted for age (years) and BMI (kg/m^2); postural tremor models were adjusted for age (years); Romberg models were adjusted for age (years), height (cm), and state; tandem gait models were adjusted for age (years) and height (cm); toe proprioception models were adjusted for age (years) and height (cm); toe vibration models were adjusted for age (years), height (cm) and state.

-- Results from models with < 5 exposed cases are not presented.

Table 4.6. Results from dose-response models for postural tremor and toe proprioception among pesticide applicators (n=678)

Lifetime days of exposure	Postural tremor (abnormal = 117, normal = 547)			Toe proprioception (abnormal = 62, normal = 603)		
	OR	95% CI	p-trend*	OR	95% CI	p-trend*
Organophosphates						
Acephate						
0	1.00			--	--	
≤ median	1.68	0.96, 2.91	0.36	--	--	
> median	1.08	0.55, 2.11		--	--	
Chlorpyrifos						
0	1.00			1.00		
≤ median	0.98	0.60, 1.59	0.76	**2.33**	**1.20, 4.54**	**0.01**
> median	1.09	0.66, 1.80		**2.38**	**1.18, 4.80**	
Coumaphos						
0 days	1.00			1.00		
≤ median	0.70	0.26, 1.83	0.95	2.16	0.90, 5.20	0.06
> median	1.16	0.54, 2.51		1.91	0.80, 4.57	
Diazinon						
0 days	1.00			1.00		
≤ median	**0.42**	**0.22, 0.79**	0.19	0.54	0.24, 1.19	0.31
> median	0.81	0.50, 1.31		0.78	0.41, 1.47	
Dichlorvos						
0 days	1.00			1.00		
≤ median	0.70	0.32, 1.53	0.59	**3.53**	**1.75, 7.13**	**<0.01**
> median	0.92	0.45, 1.89		1.99	0.87, 4.52	
Dimethoate						
0 days	1.00			--	--	
≤ median	1.84	0.75, 4.50	**0.05**	--	--	
> median	1.95	0.86, 4.41		--	--	
Disulfoton						
0 days	1.00			--	--	
≤ median	**2.33**	**1.11, 4.89**	**0.02**	--	--	
> median	1.76	0.97, 3.19		--	--	
Ethoprop						
0 days	1.00			--	--	
≤ median	1.94	0.95, 3.97	**<0.01**	--	--	
> median	**2.31**	**1.30, 4.09**		--	--	
Fonofos						
0 days	1.00			1.00		
≤ median	1.07	0.59, 1.91	0.79	**2.77**	**1.41, 5.44**	**<0.01**
> median	1.07	0.60, 1.89		**3.37**	**1.76, 6.48**	
Malathion						
0 days	1.00			1.00		
≤ median	0.76	0.44, 1.31	0.82	1.08	0.53, 2.20	0.99
> median	1.00	0.59, 1.69		1.01	0.50, 2.07	
Parathion						
0 days	1.00			--	--	
≤ median	0.65	0.31, 1.38	0.62	--	--	
> median	1.32	0.73, 2.37		--	--	

Table 4.6. Continued.

Lifetime days of exposure	Postural tremor (abnormal = 117, normal = 547)			Toe proprioception (abnormal = 62, normal = 603)		
	OR	95% CI	p-trend*	OR	95% CI	p-trend*
Organophosphates						
Phorate						
0 days	1.00		0.41	1.00		
≤ median	0.75	0.37, 1.54		0.95	0.36, 2.53	**0.02**
> median	0.83	0.50, 1.38		**2.01**	**1.13, 3.58**	
Phosmet						
0 days	--	--		1.00		
≤ median	--	--		1.66	0.61, 4.53	**<0.01**
> median	--	--		**4.14**	**1.91, 9.00**	
Tebupirimfos						
0 days	1.00			--	--	
≤ median	1.68	0.70, 4.05	**<0.01**	--	--	
> median	**2.74**	**1.23, 6.10**		--	--	
Terbufos						
0 days	1.00			1.00		
≤ median	0.90	0.56, 1.45	0.57	0.93	0.48, 1.80	0.13
> median	0.87	0.51, 1.49		1.71	0.91, 3.21	
Tetrachlorvinphos						
0 days	1.00			--	--	
≤ median	1.91	0.67, 5.43	0.52	--	--	
> median	1.14	0.51, 2.56		--	--	
Carbamates						
Aldicarb						
0 days	1.00			--	--	
≤ median	1.25	0.64, 2.44	0.19	--	--	
> median	1.51	0.78, 2.94		--	--	
Benomyl						
0 days	1.00			--	--	
≤ median	1.54	0.78, 3.03	0.62	--	--	
> median	1.01	0.49, 2.10		--	--	
Carbaryl						
0 days	1.00			1.00		
≤ median	1.26	0.75, 2.10	0.10	0.68	0.38, 1.23	**<0.01**
> median	1.52	0.92, 2.50		**0.24**	**0.11, 0.52**	
Carbofuran						
0 days	1.00			1.00		
≤ median	0.64	0.35, 1.15	0.61	1.13	0.55, 2.32	0.09
> median	1.22	0.76, 1.96		1.73	0.94, 3.17	

NOTE: OR = odds ratio; 95% CI = 95% confidence interval; postural tremor models were adjusted for age (years); toe proprioception models were adjusted for age (years) and height (cm)

* Based on the chi-square test for trend

-- Results from models with < 5 exposed cases are not presented.

Table 4.7. Adjusted associations between electrophysiological tests and pesticide use (ever-use and $\log_{10}$ lifetime days of use) among licensed pesticide applicators (n=678)

Exposure	Distal motor amplitude (mV) (n=664)		Distal motor latency (ms) * (n=655)		Nerve conduction velocity (m/s) (n=652)		Short F-wave latency (ms)* (n=544)	
	β	95% CI	β	95% CI	β	95% CI	β	95% CI
Organophosphates								
Acephate								
Ever-use	0.01	-0.53, 0.54	0.03	-0.14, 0.20	0.42	-0.42, 1.27	**1.18**	**0.20, 2.16**
Lifetime days	0.05	-0.24, 0.34	0.02	-0.07, 0.11	0.22	-0.24, 0.68	**0.73**	**0.21, 1.25**
Chlorpyrifos								
Ever-use	0.05	-0.33, 0.43	-0.07	-0.20, 0.05	0.12	-0.48, 0.73	0.09	-0.60, 0.79
Lifetime days	-0.01	-0.23, 0.21	-0.01	-0.08, 0.06	0.20	-0.16, 0.55	0.09	-0.31, 0.50
Coumaphos								
Ever-use	-0.09	-0.64, 0.46	-0.04	-0.22, 0.13	0.35	-0.53, 1.24	0.36	-0.64, 1.37
Lifetime days	-0.06	-0.45, 0.33	-0.02	-0.15, 0.10	0.24	-0.39, 0.88	-0.02	-0.71, 0.68
Diazinon								
Ever-use	0.04	-0.34, 0.42	**-0.12**	**-0.24, 0.00**	0.07	-0.54, 0.68	0.09	-0.61, 0.80
Lifetime days	0.08	-0.17, 0.33	**-0.07**	**-0.15, 0.00**	0.07	-0.33, 0.46	0.14	-0.32, 0.60
Dichlorvos								
Ever-use	-0.31	-0.82, 0.20	-0.15	-0.32, -0.01	-0.14	-0.96, 0.67	-0.66	-1.57, 0.26
Lifetime days	-0.04	-0.28, 0.40	-0.06	-0.13, 0.02	-0.11	-0.50, 0.28	-0.29	-0.72, 0.14
Dimethoate								
Ever-use	0.22	-0.40, 0.86	-0.02	-0.21, 0.18	-0.07	-1.07, 0.92	-0.04	-1.20, 1.12
Lifetime days	0.10	-0.33, 0.52	-0.01	-0.15, 0.12	-0.01	-0.68, 0.66	-0.08	-0.86, 0.70
Disulfoton								
Ever-use	0.03	-0.53, 0.59	0.07	-0.11, 0.25	-0.40	-1.30, 0.49	0.57	-0.47, 1.61
Lifetime days	0.01	-0.35, 0.37	0.03	-0.09, 0.14	-0.22	-0.80, 0.36	0.46	-0.21, 1.14
Ethoprop								
Ever-use	-0.41	-0.95, 0.12	-0.08	-0.25, 0.09	-0.60	-1.45, 0.24	-0.01	-1.01, 0.99
Lifetime days	-0.28	-0.62, 0.07	-0.06	-0.17, 0.05	-0.36	-0.91, 0.19	-0.08	-0.72, 0.57
Fonofos								
Ever-use	0.05	-0.40, 0.50	-0.09	-0.24, 0.05	0.09	-0.63, 0.81	-0.09	-0.91, 0.73
Lifetime days	0.06	-0.21, 0.33	-0.04	-0.13, 0.04	-0.03	-0.46, 0.40	-0.05	-0.54, 0.44
Malathion								
Ever-use	0.28	-0.16, 0.72	-0.02	-0.16, 0.12	-0.09	-0.79, 0.61	-0.70	-1.51, 0.11
Lifetime days	-0.03	-0.26, 0.19	-0.04	-0.11, 0.03	0.00	-0.36, 0.37	-0.28	-0.70, 0.14
Parathion								
Ever-use	0.22	-0.25, 0.68	-0.07	-0.22, -0.08	-0.01	-0.75, 0.73	0.31	-0.53, 1.15
Lifetime days	0.24	-0.04, 0.54	0.01	-0.08, 0.10	0.03	-0.43, 0.49	0.17	-0.34, 0.68
Phorate								
Ever-use	**0.47**	**0.06, 0.88**	0.01	-0.13, 0.14	0.64	-0.01, 1.29	**0.91**	**0.16, 1.66**
Lifetime days	**0.28**	**0.04, 0.53**	0.02	-0.06, 0.09	**0.50**	**0.12, 0.89**	**0.72**	**0.28, 1.16**
Phosmet								
Ever-use	-0.07	-0.63, 0.49	-0.05	-0.23, 0.13	-0.21	-1.11, 0.69	-0.27	-1.30, 0.75
Lifetime days	-0.09	-0.43, 0.25	-0.01	-0.13, 0.10	-0.10	-0.65, 0.46	-0.04	-0.68, 0.59
Tebupirimfos								
Ever-use	**0.71**	**0.05, 1.37**	0.06	-0.15, 0.27	0.78	-0.28, 1.84	-0.05	-1.24, 1.13
Lifetime days	**0.43**	**0.02, 0.84**	0.04	-0.09, 0.17	0.51	-0.14, 1.17	0.08	-0.65, 0.80
Terbufos								
Ever-use	0.20	-0.18, 0.59	0.05	-0.07, 0.17	0.21	-0.41, 0.82	0.16	-0.54, 0.86
Lifetime days	0.15	-0.06, 0.36	0.04	-0.03, 0.11	0.18	-0.15, 0.51	0.29	-0.08, 0.67

Table 4.7. Continued.

Exposure	Distal motor amplitude (mV) (n=664)		Distal motor latency (ms) * (n=655)		Nerve conduction velocity (m/s) (n=652)		Short F-wave latency (ms)* (n=544)	
	β	95% CI	β	95% CI	β	95% CI	β	95% CI
Tetrachlorvinphos								
Ever-use	-0.40	-1.03, 0.24	-0.05	-0.24, 0.15	-0.04	-1.06, 0.99	-0.36	-1.50, 0.77
Lifetime days	-0.23	-0.62, 0.16	-0.02	-0.14, 0.10	0.15	-0.47, 0.77	-0.13	-0.81, 0.56
Carbamates								
Aldicarb								
Ever-use	0.30	-0.22, 0.83	-0.06	-0.22, 0.11	-0.61	-0.23, 1.44	**1.73**	**0.74, 2.71**
Lifetime days	0.18	-0.13, 0.49	-0.06	-0.16, 0.04	0.27	-0.22, 0.77	**0.88**	**0.30, 1.45**
Benomyl								
Ever-use	0.28	-0.26, 0.82	-0.06	-0.23, 0.11	0.62	-0.23, 1.48	0.60	-0.40, 1.60
Lifetime days	0.10	-0.25, 0.46	-0.07	-0.19, 0.04	0.45	-0.11, 1.01	0.40	-0.27, 1.06
Carbaryl								
Ever-use	-0.10	-0.54, 0.33	-0.13	-0.26, 0.01	-0.30	-0.99, 0.40	-0.62	-1.40, 0.16
Lifetime days	0.11	-0.14, 0.36	**-0.10**	**-0.17, -0.02**	0.03	-0.37, 0.43	-0.30	-0.75, 0.15
Carbofuran								
Ever-use	-0.03	-0.41, 0.35	-0.07	-0.20, 0.04	-0.18	-0.78, 0.43	-0.30	-0.99, 0.40
Lifetime days	-0.01	-0.25, 0.23	-0.04	-0.11, 0.04	0.03	-0.35, 0.41	-0.05	-0.49, 0.40
Summary variables								
Lifetime days to ALL OPs	0.22	-0.05, 0.50	-0.01	-0.10, 0.08	0.31	-0.13, 0.75	0.50	-0.02, 1.01
Lifetime days to ALL pesticides	0.19	-0.19, 0.58	**-0.10**	**-0.22, -0.02**	0.53	-0.08, 1.14	**0.75**	**0.01, 1.50**
HPEEs (ever)	**0.54**	**0.10, 0.98**	-0.01	-0.15, 0.13	0.02	-0.68, 0.72	0.16	-0.63, 0.96

NOTE: HPEEs = high pesticide exposure events; Electrophysiological tests were adjusted for age (years), height (cm), foot temperature (°C), and state

* Scores have been inverted so that lower scores indicate poorer test results.

Table 4.8. Adjusted associations for quantitative functional PNS tests and pesticide use (ever-use and $\log_{10}$ lifetime days of use) among licensed pesticide applicators (n=678)

Exposure	Hand strength z-score (n=671)		Sway speed, eyes open (mm/s) * (n=655)		Sway speed, eyes closed (mm/s) * (n=656)		Vibrotactile threshold, (log μ) * (n=667)	
	β	95% CI	β	95% CI	β	95% CI	β	95% CI
Organophosphates								
Acephate								
Ever-use	0.10	-0.05, 0.24	0.53	-0.40, 1.46	1.05	-0.82, 2.93	0.01	-0.06, 0.08
Lifetime days	0.05	-0.03, 0.13	0.31	-0.19, 0.82	0.48	-0.54, 1.50	0.00	-0.04, 0.04
Chlorpyrifos								
Ever-use	0.10	-0.05, 0.24	0.53	-0.40, 1.46	1.05	-0.82, 2.93	0.01	-0.06, 0.08
Lifetime days	0.03	-0.03, 0.09	-0.23	-0.61, 0.16	-0.41	-1.18, 0.36	0.01	-0.02, 0.05
Coumaphos								
Ever-use	0.00	-0.15, 0.15	0.47	-0.48, 1.42	0.10	-1.82, 2.02	0.00	-0.09, 0.09
Lifetime days	0.01	-0.10, 0.12	0.25	-0.43, 0.93	0.36	-1.01, 1.74	-0.01	-0.07, 0.06
Diazinon								
Ever-use	0.03	-0.07, 0.14	-0.03	-0.70, 0.64	0.14	-1.21, 1.49	-0.01	-0.07, 0.05
Lifetime days	0.03	-0.04, 0.10	0.13	-0.30, 0.57	0.36	-0.52, 1.24	0.00	-0.04, 0.04
Dichlorvos								
Ever-use	-0.02	-0.16, 0.12	0.19	-0.70, 1.08	-0.84	-2.64, 0.96	-0.03	-0.11, 0.05
Lifetime days	0.02	-0.10, 0.04	0.18	-0.24, 0.61	-0.18	-1.04, 0.67	-0.01	-0.04, 0.02
Dimethoate								
Ever-use	0.06	-0.11, 0.24	-0.27	-1.37, 0.83	-0.49	-2.71, 1.72	0.04	-0.07, 0.14
Lifetime days	0.02	-0.10, 0.13	-0.29	-1.03, 0.45	-0.71	-2.20, 0.78	0.03	-0.03, 0.10
Disulfoton								
Ever-use	0.08	-0.07, 0.23	-0.08	-1.05, 0.90	-1.06	-3.02, 0.90	0.06	-0.02, 0.14
Lifetime days	0.04	-0.06, 0.14	-0.05	-0.68, 0.57	-0.88	-2.14, 0.37	0.02	-0.03, 0.07
Ethoprop								
Ever-use	-0.03	-0.17, 0.12	-0.50	-1.44, 0.44	-0.83	-2.72, 1.07	-0.02	-0.10, 0.06
Lifetime days	-0.02	-0.11, 0.08	-0.35	-0.96, 0.25	-0.60	-1.81, 0.61	-0.01	-0.06, 0.04
Fonofos								
Ever-use	0.03	-0.09, 0.16	-0.29	-1.08, 0.50	-0.09	-1.69, 1.51	-0.02	-0.09, 0.04
Lifetime days	0.02	-0.05, 0.10	-0.14	-0.61, 0.34	0.02	-0.94, 0.97	0.01	-0.03, 0.04
Malathion								
Ever-use	0.10	-0.02, 0.22	-0.08	-0.86, 0.69	0.53	-1.03, 2.09	-0.02	-0.09, 0.05
Lifetime days	0.05	-0.01, 0.11	-0.24	-0.64, 0.16	-0.26	-1.07, 0.54	-0.02	-0.06, 0.01
Parathion								
Ever-use	0.04	-0.08, 0.17	**1.09**	**0.29, 1.89**	1.57	-0.05, 3.19	0.01	-0.07, 0.08
Lifetime days	0.00	-0.08, 0.08	**0.71**	**0.21, 1.21**	0.92	-0.09, 1.93	0.01	-0.04, 0.06
Phorate								
Ever-use	-0.05	-0.16, 0.06	0.31	-0.41, 1.04	0.72	-0.74, 2.19	0.04	-0.02, 0.10
Lifetime days	-0.03	-0.09, 0.04	0.13	-0.30, 0.56	0.42	-0.45, 1.29	0.03	-0.01, 0.06
Phosmet								
Ever-use	**0.23**	**0.08, 0.39**	-0.34	-1.33, 0.64	0.52	-1.45, 2.49	-0.06	-0.15, 0.03
Lifetime days	**0.15**	**0.05, 0.24**	-0.34	-0.93, 0.26	0.06	-1.14, 1.26	-0.04	-0.10, 0.01
Tebupirimfos								
Ever-use	-0.09	-0.28, 0.09	**1.40**	**0.24, 2.56**	1.19	-1.16, 3.53	0.04	-0.07, 0.14
Lifetime days	-0.05	-0.16, 0.06	**0.82**	**0.10, 1.53**	0.73	-0.72, 2.18	0.03	-0.03, 0.09
Terbufos								
Ever-use	0.06	-0.04, 0.17	0.05	-0.73, 0.62	-1.07	-2.43, 0.29	0.02	-0.04, 0.08
Lifetime days	0.03	-0.03, 0.09	-0.10	-0.47, 0.26	-0.58	-1.32, 0.15	0.00	-0.03, 0.03

Table 4.8. Continued.

Exposure	Hand strength z-score (n=671)		Sway speed, eyes open (mm/s) * (n=655)		Sway speed, eyes closed (mm/s) * (n=657)		Vibrotactile threshold, (log μ) * (n=667)	
	β	95% CI	β	95% CI	β	95% CI	β	95% CI
Tetrachlorvinphos								
Ever-use	0.10	-0.08, 0.27	-0.19	-1.32, 0.94	-0.98	-3.26, 1.31	-0.01	-0.11, 0.09
Lifetime days	0.06	-0.05, 0.17	-0.09	-0.78, 0.61	-0.39	-1.79, 1.01	0.00	-0.06, 0.06
Carbamates								
Aldicarb								
Ever-use	-0.01	-0.16, 0.13	**0.99**	**0.07, 1.91**	**2.47**	**0.62, 4.32**	**0.11**	**0.04, 0.19**
Lifetime days	-0.00	-0.09, 0.08	**0.63**	**0.08, 1.17**	**1.16**	**0.07, 2.26**	**0.05**	**0.01, 0.10**
Benomyl								
Ever-use	0.04	-0.10, 0.19	0.61	-0.33, 1.54	1.39	-0.50, 3.28	0.04	-0.04, 0.12
Lifetime days	-0.01	-0.10, 0.09	0.33	-0.29, 0.95	0.33	-0.90, 1.57	0.02	-0.03, 0.08
Carbaryl								
Ever-use	0.04	-0.08, 0.16	-0.43	-1.18, 0.32	0.23	-1.30, 1.76	-0.04	-0.10, 0.03
Lifetime days	0.06	-0.01, 0.12	-0.15	-0.59, 0.29	0.31	-0.57, 1.19	-0.01	-0.04, 0.03
Carbofuran								
Ever-use	0.04	-0.06, 0.15	0.27	-0.39, 0.93	0.17	-1.17, 1.50	0.02	-0.04, 0.08
Lifetime days	0.04	-0.03, 0.11	0.03	-0.39, 0.45	-0.27	-1.11, 0.58	0.02	-0.02, 0.06
Summary variables								
Lifetime days to ALL OPs	0.01	-0.07, 0.08	0.14	-0.34, 0.62	0.14	-0.83, 1.11	0.01	-0.03, 0.05
Lifetime days to ALL pesticides	0.07	-0.04, 0.17	0.22	-0.45, 0.89	0.29	-1.06, 1.64	0.03	-0.03, 0.09
HPEEs (ever)	**0.14**	**0.02, 0.26**	0.37	-0.40, 1.14	0.96	-0.60, 2.51	0.01	-0.06, 0.08

NOTE: HPEEs = high pesticide exposure events; Hand strength models were adjusted for age (years), BMI (kg/m^2), height (cm) and state; sway speed models (with both eyes open and closed) were adjusted for age (years), height (cm) and state; vibrotactile threshold models were adjusted for age (years) and height (cm)

* Scores have been inverted so that lower scores indicate poorer test performance.

CHAPTER 5

CONCLUSIONS

Study Summary

The goal of this research was to examine associations between long-term pesticide exposures and neurological outcomes among pesticide applicators. Specifically, we examined: 1) associations between estimates of cumulative lifetime exposure to organophosphate (OP) pesticides and neurobehavioral measures of central nervous system (CNS) function, 2) associations between high pesticide exposure events (HPEEs) and neurobehavioral measures of CNS function, and 3) associations between estimates of cumulative lifetime exposure to OP pesticides and measures of peripheral nervous system (PNS) function.

To achieve these aims, a battery of neurological tests was administered to 701 licensed pesticide applicators enrolled in the Agricultural Health Study (AHS) in Iowa and North Carolina. Detailed information about individual pesticide exposure and covariates were obtained from AHS interviews and a questionnaire administered at the time of neurological testing. Associations between pesticide use and neurological outcome measures were estimated with linear and logistic regression methods, controlling for age and outcome-specific covariates. Overall, we observed significant associations between a few individual pesticides and measures of CNS and PNS function. We also observed significant associations between and HPEEs and measures of CNS function.

Main Findings

Associations between estimates of cumulative lifetime exposure to organophosphate (OP) pesticides and neurobehavioral measures of central nervous

system function (Chapter 2): (i) Ever-use and lifetime days of ethoprop use was significantly associated with adverse performance on a test of motor speed and visual scanning (Sequences A), (ii) ever-use and lifetime days of malathion use was significantly associated with adverse performance on a test of visual scanning and processing (Digit-Symbol), (iii) ever-use and lifetime days of chlorpyrifos use was significantly associated with better performance on tests of motor coordination (Grooved Pegboard) and verbal learning and memory (HVLT Delayed Recall), (iv) ever-use and lifetime days of coumaphos and tetrachlorvinphos were both associated with better verbal learning and memory (HVLT Total Recall), (v) significant interaction by state of residence was observed for a few OP pesticides (i.e. chlorpyrifos, coumaphos, malathion, and all OP pesticides) and neurobehavioral outcomes. Overall, our results do not provide strong evidence that long-term OP pesticide use, in the absence of previous pesticide poisoning, is associated with adverse neurobehavioral test performance.

Associations between high pesticide exposure events (HPEEs) and neurobehavioral measures of central nervous system function (Chapter 3): (i) Participants with HPEEs performed significantly poorer on a test of visual scanning and processing (Digit-Symbol) than those without HPEEs, (ii) participants with HPEEs performed significantly poorer on a test of motor speed and scanning (Sequences A) than those without HPEEs. Small but meaningful associations were observed between HPEEs and adverse CNS outcomes suggesting that high-level pesticide exposures that do not result in overt poisonings may contribute to adverse long-term neurological health effects. To our knowledge, this is the first study to examine the association between unusually high pesticide exposure events and neurobehavioral function among agricultural workers. Consequently, the results of this study fill a gap in knowledge about the long-term neurobehavioral effects of pesticide exposure levels between low-level exposure and overt high level exposure resulting in pesticide poisoning.

Associations between estimates of cumulative lifetime exposure to OP pesticides and measures of peripheral nervous system function (Chapter 3): (i) Abnormal physical examination tests were significantly associated with ever-use of 10 OP pesticides, (ii) toe proprioception was the most sensitive neurological physical examination test and was adversely associated with ever-use of six OP pesticides (chlorpyrifos, coumaphos, dichlorvos, fonofos, phosmet, and tetrachlorvinphos); a monotonic increase in odds ratios was observed for three of these chemicals (chlorpyrifos, fonofos and phosmet), (iii) postural tremor was a sensitive test and was adversely associated with ever-use of four OP pesticides (dimethoate, disulfoton, ethoprop and tebupirmiphos); a monotonic increase in odds ratios was observed for dimethoate, ethorop and tebupirmiphos, (iv) inverse associations were observed for ever-use of three OP pesticides and all four carbamate pesticides and neurological physical examination tests, (v) mostly null associations were observed between OP pesticide use and quantitative tests of PNS function (i.e. electrophysiological tests, hand strength, sway speed and vibrotactile threshold).

Our finding of mostly null associations for the quantitative measures of PNS function is consistent with other studies [34-36]. However, unlike previous investigations, we observed several significant associations between long-term OP pesticide use and abnormal neurological physical examination results. Most notably, toe proprioception and postural tremor were adversely associated with long-term use of several OP pesticides. These tests may be more sensitive indicators of peripheral nerve impairment associated with pesticide use.

Strengths

An important strength of this research was the size of the study sample. We administered neurobehavioral and neurological tests to 701 pesticide applicators in Iowa and North Carolina. The large size of the sample allowed for greater statistical power

than the majority of studies published to date. Furthermore, because the study sample included pesticide applicators from two distinct geographical locations with different crops and farming practices, the results of this research may be relevant to a large segment of the farming population.

An additional strength of this research is the use of detailed pesticide exposure information from the AHS. In most previous investigations (reviewed in Chapter 1), pesticide exposure was often represented by a dichotomous variable regardless of agent(s) or exposure duration; few studies examined long-term exposure to specific pesticides. Exposure estimation using dichotomized exposure metrics results in the pooling of a wide range of actual exposures (agents and durations of exposure) into a single exposed group. As a result, pesticide exposure information is lost and observed associations may underestimate true associations. In the present research, we examined both ever-use and cumulative lifetime use of 20 individual pesticides (16 OPs and four carbamates). This information has been periodically updated by the AHS since 1993 and represents true prospective pesticide exposure information. Furthermore, pesticide information has been validated by assessment of its reliability and plausibility [62-64].

In addition, we examined neurological and neurobehavioral health effects of pesticide exposure with a reliable, validated and widely-used battery of tests assessing a range of neurological domains to assure that high quality health outcome information was obtained. All of the tests used in this research have well established psychometric properties and have been used extensively in previous investigations of neurotoxicant-exposed individuals.

Potential Limitations

There are a few important limitations of this research worth noting. First, our overall response rate was modest. Of the 1,807 AHS participants eligible to participate in this research, 701 (39%) were enrolled and completed neurological testing. Efforts were

made to enhance response rates. We mailed introductory letters and called eligible participants to provide a detailed description of the project. A reminder letter was mailed to those who agreed to participate and a reminder call was made within a few days of their scheduled appointment. Neurological testing was conducted at two locations within each state. These sites were selected based on the number of AHS participants living within a 150 mile radius in order to minimize study participant travel. Neurological testing was conducted during the winter months, which is generally the slowest time for farmers. Furthermore, participants were reimbursed for time and travel expenses. Despite these efforts, the response rate was lower than we had anticipated.

If non-response was independent of both pesticide exposure and neurological outcomes, then we would not expect a bias in the exposure-effect association. Whereas, if non-response was dependent on exposure and outcome simultaneously, then the observed association might either overestimate or underestimate the true association. Because neurological function was not measured among non-participants, there is no direct way of knowing the direction or magnitude of the bias. However, we did have demographic and lifestyle information and could assess whether participants in our sample differed from the AHS cohort on these variables. Overall, non-participation was higher in North Carolina than in Iowa and non-participants were less educated than participants. Furthermore, participants were similar to non-participants on important attributes such as age, and total lifetime days of pesticide use, suggesting comparability between them. Although possible, we have no basis to conclude that the exposure-effect association among study participants was substantially different than the exposure-effect association among non-participants.

Another limitation of this research is that our study sample may have been highly selected. We randomly sampled individuals from the AHS cohort, however, only those who had completed all phases of AHS interviews were eligible and we excluded individuals with a number of health conditions. As such, it is possible that AHS

participants who left farming prior to our study or who were unable to complete all of the AHS questionnaires may have been more affected by pesticide exposure than those who were eligible and participated in the current study. As a result of such selective survival and of the exclusion criteria that were applied, it is possible that we enriched our study sample with the least susceptible members of the exposed population. Consequently, associations observed among members of this sample may underestimate associations that occur among all exposed members of the population.

Finally, we cannot evaluate the temporal relationship between long-term pesticide exposure and adverse neurological function. Although most of the pesticide exposure information was collected prospectively prior to neurological testing, it is possible that some individuals had deficits in neurological function prior to exposure to any pesticides. Regardless, we are unaware of any mechanism that would select those with poorer neurological function for greater pesticide exposure.

Public Health Implications

Given the widespread use of OP and other agricultural pesticides in the US and worldwide, a large number of workers are potentially exposed to these chemicals. Consequently, the adverse health effects of pesticides are a major public health concern.

The present research adds to the growing body of evidence that long-term pesticide exposure, at levels insufficient to cause clinically apparent toxicity, may result in persistent neurological deficits. Although pesticide applicators are usually required to wear personal protective equipment (e.g. gloves, masks, protective clothing) when handling pesticides, compliance to these safety requirements is often low [85-88]. Other factors can also increase the probability of exposure to pesticides including inadequate safety training and improper work practices (e.g. failure to wash hands or changing clothing following pesticide application). Increased efforts aimed at reducing pesticide

exposure and preventing high pesticide exposure events should be a public health priority.

Directions for Future Research

Few studies have examined the neurological effects of individual OP pesticides. As discussed previously, most investigations have used dichotomized exposure metrics based on job title or occupation. Our research is unique in that we examined the association between long-term exposure to 16 individual OP pesticides and neurological outcome measures. Although, overall, we observed mostly null associations, we did observe significant adverse associations with both ever-use and lifetime days of use to a few individual OP pesticides. Consequently, additional epidemiological studies examining specific chemicals, rather than pooling all exposures into a dichotomized exposure metric, are merited.

In the present research, we observed several significant adverse associations between pesticide use and neurological physical examination results, but not for the analogous quantitative measures (Chapter 4). Although it is unclear why these differences were observed, it is possible that the physical examination better captures clinically relevant peripheral nerve impairment than do the quantitative measures. Most of the previous studies examining peripheral nerve function (reviewed in Chapter 1) did not include a standard neurological physical examination [31, 34-36, 38]. If, in fact, physical examination is a more sensitive indicator of peripheral nerve impairment, then future studies should include it in their batteries of neurological evaluation methods.

Emerging research suggests that individual susceptibility to OP pesticide exposure may be influenced by polymorphisms in genes affecting pesticide metabolism. Metabolism of OP pesticides, for example, is influenced by several genes such as paraoxonase (PON1) and the cytochrome P450s. Because the role of genetic variation in pesticide neurotoxicity is a relatively new area of research, studies examining the effect

of genetic polymorphisms on the relationship between pesticide exposures and neurological and neurobehavioral outcomes (*i.e.*, effect measure modification) are needed.

Conclusion

In this large, epidemiological study of licensed pesticide applicators, we examined the association between long-term pesticide exposure and neurological outcomes. Our results suggest that exposure to a few individual OP pesticides and high pesticide exposure events may contribute to adverse central and peripheral nervous system function. The observed exposure-effect associations remained after adjustment for confounding and were independent of past-diagnosed pesticide poisoning. In summary, we believe this research contributes important new evidence to an inconsistent literature, and adds valuable insight into the neurological health effects of long-term exposure to OP pesticides.

APPENDIX: COMPARATIVE VALUES FOR NEUROBEHAVIORAL AND NEUROLOGICAL TESTS

Table A.1. Comparative values for neurobehavioral tests

Test	Reference	Study population/mean age	N	Mean	SD
Continuous Performance Test (ms)	Present Study	Male pesticide applicators/61 yrs	657	426.5	43.8
	Tsai, 1997 [89]	Unexposed, male paint manufacturing workers/38yrs	47	479.1	78.5
	Letz, 1996 [90]	Male, U.S. Army Veterans/40 yrs	757	352.2	35.7
Digit-Symbol (s)	Present Study	Male pesticide applicators/61 yrs	658	117.3	22.8
	White, 2003 [57]	Non-cognitively impaired males (85%) and females (15%)/46 yrs	66	91.5	20.9
	Letz, 2003 [13]	Male (50%) and female (50%) outpatients of an epilepsy and neurology clinic/44 yrs	299	137.8	40.4
Finger tapping, dominant hand (# of taps)	Present Study	Male pesticide applicators/61 yrs	662	53.6	9.6
	White, 2003 [57]	Non-cognitively impaired males (85%) and females (15%)/46 yrs	66	71.9	10.2
	Tsai, 1997 [89]	Unexposed, male paint manufacturing workers/38yrs	47	69.4	29.1

Table A.1. Continued.

Test	Reference	Study population/mean age	N	Mean	SD
Grooved pegboard, dominant hand (s)	Present Study	Male pesticide applicators/61 yrs	665	91.3	23.5
	Letz, 2000 [52]	Unexposed male industrial plant workers/71 yrs	82	93.5	25.2
	Letz, 1996 [90]	Male, U.S. Army Veterans/40 yrs	738	71.6	11.0
Hopkins Verbal Learning, Delayed Recall (#correct)	Present Study	Male pesticide applicators/61 yrs	662	6.7	2.8
	Letz, 2003 [13]	Male (50%) and female (50%) outpatients of an epilepsy and neurology clinic/44 yrs	311	6.9	3.3
Sequences A (s)	Present Study	Male pesticide applicators/61 yrs	650	42.8	14.6
	Letz, 2003 [13]	Male (50%) and female (50%) outpatients of an epilepsy and neurology clinic/44yrs	300	26.6	11.8
	Letz, 2000 [52]	Unexposed male industrial plant workers/71 yrs	83	41.8	16.3
Sequences B (s)	Present Study	Male pesticide applicators/61 yrs	640	64.1	21.0
	Letz, 2003 [13]	Male (50%) and female (50%) outpatients of an epilepsy and neurology clinic/44 yrs	297	47.8	24.2
	White, 2003 [57]	Non-cognitively impaired males (85%) and females (15%)/46 yrs	66	42.1	19.2
	Letz, 2000 [52]	Unexposed male industrial plant workers/71 yrs	83	90.5	32.0

Table A.2. Comparative values for electrophysiological tests of the peroneal motor nerve

Test	Reference	Study population/mean age	N	Mean	SD
Distal motor amplitude (mV)	Present Study	Male pesticide applicators/61 yrs	665	4.9	2.6
	Frumkin, 2001 [91]	Unexposed male (89%) and female (11%) workers/49 years	97	6.3	3.0
	Letz, 2000 [52]	Unexposed male industrial plant workers/71 yrs	80	3.7	2.3
	Letz, 1994 [42]	Male, U.S. Army Veterans/38 yrs	4,017	6.9	3.0
Distal motor latency (ms)	Present Study	Male pesticide applicators/61 yrs	656	5.1	0.9
	Albers, 2007 [38]	Male and female Saran-manufacturing workers/41 years	60	4.5	0.6
Nerve conduction velocity (m/s)	Present Study	Male pesticide applicators/61 yrs	653	44.1	4.4
	Frumkin, 2001 [91]	Unexposed male (89%) and female (11%) workers/49 years	95	43.6	3.7
	Letz, 2000 [52]	Unexposed male industrial plant workers/71 yrs	78	43.1	4.1
	Letz, 1994 [42]	Male, U.S. Army Veterans/38 yrs	4,016	46.4	4.1
Short F-wave latency (ms)	Present Study	Male pesticide applicators/61 yrs	540	53.0	5.0
	Letz, 2000 [52]	Unexposed male industrial plant workers/71 yrs	67	53.8	4.5
	Frumkin, 2001 [91]	Unexposed male (89%) and female (11%) workers/49 years	84	50.5	5.0

Table A.3. Comparative values for hand strength dynamometry tests

			Dominant hand			Non-dominant hand		
Test	Reference	Study population/mean age	N	Mean	SD	N	Mean	SD
Grip strength (kg)	Present Study	Male pesticide applicators/61 yrs	666	38.2	9.6	668	38.2	9.8
	Charles, 2006 [92]	Male, Japanese-American Workers /53 yrs	3,519	39.6	6.1	--	--	--
	Dixon, 2005 [93]	Healthy males/age 55-65years	180	44.2	7.9	180	42.6	7.0
	Letz, 2000 [52]	Unexposed male industrial plant workers/71 yrs	84	40.4	8.1	--	--	--
Key pinch (kg)	Present Study	Male pesticide applicators/61 yrs	665	10.9	2.0	667	10.8	2.1
	Letz, 2000 [52]	Unexposed male industrial plant workers/71 yrs	84	9.5	2.0	--	--	--
Palmer pinch (kg)	Present Study	Male pesticide applicators/61 yrs	665	9.9	2.3	666	10.1	2.4
	Letz, 2000 [52]	Unexposed male industrial plant workers/71 yrs	84	8.9	2.0	--	--	--

Table A.4. Comparative values for sway speed and vibrotactile thresholds

Test	Reference	Study population/mean age	N	Mean	SD
Sway speed-eyes open (mm/s)	Present Study	Male pesticide applicators/61 yrs	655	14.6	5.1
	Letz, 2000 [52]	Unexposed male industrial plant workers/71 yrs	78	10.1	5.0
	Letz, 1996 [94]	Male construction workers/52 years	114	13.8	4.1
Sway speed-eyes closed (mm/s)	Present Study	Male pesticide applicators/61 yrs	656	22.9	10.2
	Letz, 2000 [52]	Unexposed male industrial plant workers/71 yrs	78	16.1	8.6
	Letz, 1996 [94]	Male construction workers/52 years	144	19.3	6.7
Vibrotactile threshold - great toe (log μ)	Present Study	Male pesticide applicators/61 yrs	660	1.5	0.5
	Letz, 2000 [52]	Unexposed male industrial plant workers/71 yrs	81	1.9	0.3
	Gerr, 1994 [43]	Male, U.S. Army Veterans/38 yrs	4,056	1.3	0.5

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