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Parkinsonism and Pesticide Exposure among Rural Residents of Washington State

by

Lawrence S. Engel

A dissertation submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

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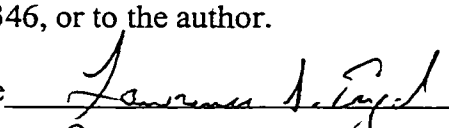
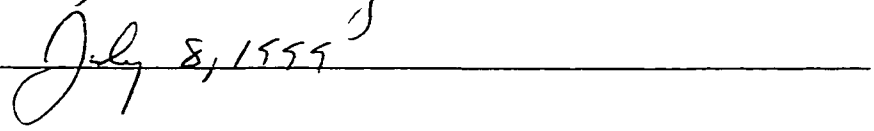
Signature 

Date 

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Abstract

Parkinsonism and Pesticide Exposure among Rural Residents of Washington State

by Lawrence S. Engel

Chairperson of the Supervisory Committee:
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Department of Epidemiology

Objective: To determine the prevalence of parkinsonism among a cohort of rural men, primarily orchardists, and to examine associations between that prevalence and smoking, occupational pesticide use, and MAO-B and ND1/4216 genetic polymorphisms. Also to assess the accuracy of pesticide use recall. *Methods:* All 323 subjects had participated during the 1970s in a cohort study of men occupationally exposed to pesticides. Subjects were given a neurologic examination and completed a questionnaire concerning lifetime occupational pesticide use. Parkinsonism was defined by the presence of two or more of resting tremor, rigidity, bradykinesia, and postural reflex impairment. Prevalence ratios were estimated for parkinsonism in relation to smoking, farming, pesticide use, genotype, and gene-environment interactions. Recall sensitivity and specificity were estimated by comparing pesticide use reported in the current study to that reported in the original study. *Results:* Mean age of subjects was 69.4 years (range: 49-96). Prevalence of slight or greater parkinsonism was 20.7% and mild or greater parkinsonism was 1.5%. A prevalence ratio of 2.0 (95% CI: 1.0, 4.2) was observed for subjects with the longest duration of general pesticide exposure; the prevalence ratio for intermediate duration was elevated but non-significant. However, we observed no increased risk associated with specific pesticides or pesticide classes, nor with a history of farming. We found no association between smoking or genotype and parkinsonism, nor any modification of

effect of smoking, pesticide exposure, or farming by either genotype. Sensitivity of pesticide recall was good to excellent (0.6-0.9) for broad categories such as insecticides, herbicides, and fungicides, and for widely used pesticides and pesticide classes, but was lower (0.1-0.6) for other pesticide categories. *Conclusion:* There was an increased risk of parkinsonism associated with long-term occupational exposure to pesticides, but we could not detect any associations between parkinsonism and specific pesticides. Parkinsonism was not associated with smoking or with polymorphisms of MAO-B intron 13 or ND1/4216, nor were there interactions between genotype and any exposure in relation to parkinsonism. The level of recall accuracy observed here is probably adequate for epidemiologic analyses of broad categories of pesticides, but is a limitation for detecting more specific associations.

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ACKNOWLEDGMENTS

I would like to give special thanks to the chairperson of my Dissertation Committee, Harvey Checkoway; the other Committee members, Matthew Keifer, William Longstreth, and Noah Seixas; and my Graduate Student Representative, Robert Myall. I also wish to thank Kent Anger, Richard Camicioli, KC Scott, and the staff people at the University of Washington and Oregon Health Sciences University who helped make this study possible. I am grateful to Mary Lou Thompson for her statistical assistance. Lastly, I would like to thank the subjects, who have generously and repeatedly volunteered their time over the years.

DEDICATION

I wish to dedicate this dissertation to my parents who always wanted a doctor in the family.

INTRODUCTION

The syndrome of parkinsonism is characterized by four signs: muscle rigidity, resting tremor, bradykinesia, and impairment of postural reflexes.[1,2] It results from a decline in level or function of the neurotransmitter dopamine in the substantia nigra and striatum.[2] Parkinsonism should be distinguished from Parkinson's disease, which is a specific parkinsonian disorder whose principal neuropathological feature is the loss of dopaminergic cells in the substantia nigra.[1,2] Parkinsonism refers to a range of clinical disorders which have some or all of the signs associated with Parkinson's disease, but which may also present signs atypical of that disease. Although this dissertation examines parkinsonism, and not Parkinson's disease specifically, it is with the intent of improving understanding at both levels.

Parkinsonism has been associated with several chemical exposures, including manganese, carbon disulfide, and other organic solvents.[1] The syndromes induced by these agents, however, are clinically distinct from Parkinson's disease (PD), leaving the etiology of PD largely a mystery. Thus, the discovery in the early 1980s that intravenous drug abusers exposed to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a contaminant of a synthetic narcotic, developed a condition remarkably similar to Parkinson's disease[3] increased attention towards possible environmental causes of PD. The subsequent recognition of structural similarity between MPTP and the herbicide paraquat[4] led several researchers to investigate agricultural exposures as possible risk factors for Parkinson's disease.[5]

The results of this research have been equivocal, with conflicting findings regarding a history of farming, pesticide use, and well water consumption in relation to PD.[4,6-30] Even among studies that have found an association with pesticide use, few have identified specific chemicals or chemical classes.[14,18,23]

Most research examining genetics in relation to Parkinson's disease has produced similarly mixed results.[31] This fact, coupled with an improving understanding of the mechanisms of detoxification and bioactivation of exogenous compounds in humans, has led to growing interest in possible gene-environment interactions in relation to PD.[31-33]

This dissertation examines parkinsonism and agricultural exposures among male residents of central Washington. It is based on a study conducted in 1997 which located and tested a group of men, primarily orchardists, who had previously participated in a prospective study investigating possible health effects of occupational pesticide exposure. That study, conducted by the Washington State Department of Health, took place from 1972 to 1976. Information gathered in both studies is used in the chapters that follow.

This dissertation is divided into four chapters. The first chapter assesses the prevalence of individual parkinsonian signs and of a parkinsonian syndrome defined by those signs. It investigates the association of parkinsonism prevalence with cigarette smoking and age, and provides a context for the subsequent chapters. The second chapter examines the relationship between parkinsonism and occupational pesticide exposure. The third chapter investigates the association between parkinsonism and certain genotypes, and considers parkinsonism in relation to interactions between pesticides and those genotypes (i.e., gene-environment interaction). The last chapter assesses the accuracy of the pesticide exposure data through sensitivity and specificity analyses comparing pesticide use reported in the follow-up study to that reported in the original study.

CHAPTER 1: PREVALENCE OF PARKINSONISM AMONG RURAL MALE RESIDENTS OF WASHINGTON STATE

ABSTRACT

Objective: To determine the prevalence of parkinsonism among rural male residents of Washington State and to examine associations between that prevalence and certain demographic and lifestyle factors. *Methods:* All 323 subjects in this study had previously participated in a cohort study of men occupationally exposed to pesticides, mostly as orchardists. Subjects were given a structured neurologic examination and completed a self-administered questionnaire which elicited detailed information on demographic characteristics, including smoking history. Parkinsonism was defined by both 1) the presence of two or more of resting tremor, rigidity, bradykinesia, and postural reflex impairment in subjects not on anti-parkinsonian medication, or the presence of one sign if they were on such medication and 2) the severity of those signs. Parkinson's disease was not studied explicitly because it could not be distinguished from other parkinsonian syndromes. A generalized linear model was used to estimate prevalence ratios for parkinsonism in relation to current and lifetime (pack-years) smoking. *Results:* Subjects had a mean age of 69.4 years (range: 49-96, standard deviation: 8.1). Prevalence of slight or greater parkinsonism was 20.7% and mild or greater parkinsonism was 1.5%. There were no cases of marked or severe parkinsonism. We observed no association between any smoking measures and parkinsonism. *Conclusion:* There was a high prevalence of slight or greater parkinsonism in this elderly male cohort of rural residents. Smoking was not related to parkinsonism. In light of the consistent findings in other studies of a seemingly protective effect of smoking on Parkinson's disease, we conclude that the lack of a protective effect in this study may be due to the mild nature of the parkinsonism observed here or to the particular factors contributing to parkinsonism in this population.

INTRODUCTION

Signs of parkinsonism are often found in older people.[34,35] The prevalence of parkinsonism has been assessed in various populations,[36-39] but most prevalence studies have been clinic-, hospital-, or record-based and, thus, may exclude a large number of persons who do not seek medical attention for these parkinsonian signs. In addition, most studies have focused only on physician-diagnosed Parkinson's disease. However, a few population-based studies have examined the prevalence of parkinsonism, including previously undiagnosed or subclinical disease. Among these, estimates in males range between 0.7-14.7% for those aged 65-74 to 4.0-53.0% for those aged 85 and older.[36-38]

The present study examines the prevalence of parkinsonism and parkinsonian signs among a cohort of men occupationally exposed to pesticides, mostly as orchardists, in central Washington State. All subjects were participants in an earlier cohort study of men occupationally exposed to pesticides which was begun in the early 1970s by the Washington State Department of Health. Subjects in the present study were given a structured neurological examination and asked to complete a detailed questionnaire covering demographics, lifestyle factors, and lifetime pesticide exposure. Parkinsonism was defined as the presence of two or more of the following signs: bradykinesia, resting tremor, rigidity, and postural reflex impairment. This study did not attempt to distinguish idiopathic Parkinson's disease from other forms of parkinsonism which can be caused by a variety of factors including toxins, neuroleptic agents, multiple-system atrophy, and vascular disease.

The purpose of this study was to determine the prevalence of parkinsonism for comparison with other prevalence studies and to provide the background for later analyses which will examine the relation between parkinsonism and pesticide exposures in these subjects.

METHODS

SUBJECTS

All subjects had previously participated in a cohort study carried out by the Washington State Department of Health from 1972 through 1976. That study examined a variety of health outcomes in relation to pesticide exposure among residents of central Washington. Exposed subjects in that study were recruited from persons engaged in occupations with substantial pesticide exposure during that time period. The study population included mostly orchardists (n=739, 56.8%), professional pesticide applicators (n=30, 2.3%), pesticide formulation plant workers (n=7, 0.5%), other farm or agricultural workers (n=6, 0.5%), and persons with no occupational pesticide exposure (n=499, 38.4%). Non-exposed subjects were frequency-matched to exposed subjects by age, race, and work regimen (i.e., degree of physical activity). Subjects were male, mostly non-Hispanic Caucasian (95.0%), and aged 18 to 88 at the time they entered that study.

Using contact information provided by subjects in the original study, as well as leads from phone books, the Washington State Department of Licensing, the Health Care Financing Administration, and the National Death Index, we attempted to ascertain the vital status and whereabouts of the 1,300 participants of that study. Four hundred sixty six subjects (35.8%) were deceased; 220 (16.9%) could not be located. Letters were sent to the remaining 614 subjects (47.2%) who were known, or believed, to be alive in the summer of 1997, inviting them to participate in the current study. Of the 482 persons who were subsequently reached by phone, 323 agreed to participate (67.0% of those reached by phone; 52.6% of those known, or believed, to be alive at that time). The exposure distribution in this group was similar to that observed in the original cohort, with 186 orchardists (57.6%), 6 professional pesticide applicators (1.9%), 2 pesticide formulation plant workers (0.6%), 3 other farm or agricultural workers (0.9%), and 125 persons with no occupational pesticide exposure (38.7%) . These subjects were then asked to come to a centrally located testing center for a 3-4 hour assessment. The study protocol was

approved by the University of Washington and Oregon Health Sciences University Human Subjects Committees, and all participants provided written, informed consent.

EXAMINATION

A neurologist-trained nurse administered an approximately 20 minute structured, uniform neurological examination to all subjects. The presence and severity of motor signs were recorded by the nurse using the United Parkinson's Disease Rating Scale (UPDRS).[40] Signs included postural, action, and resting tremors, rigidity in each limb, postural reflex impairment, bradykinesia, as well as speech impairment, hypomimia, and slowness of: finger taps (repeated tapping of thumb with index finger), hand movements (repeated opening and closing of hands), rapid alternating movements (repeated pronation and supination of hands), rising from a chair, and gait.

WORK HISTORY ASSESSMENT

Years of employment in farming or other pesticide-related occupations, as well as detailed occupational exposure information, was assessed via a self-administered questionnaire taken at the time of the neurological examination. Demographic information such as age and race, health conditions (including diseases of the blood, cancer, and nervous, respiratory, cardiovascular, gastrointestinal, endocrine, musculoskeletal, and urogenital systems), use of medications, and history of alcohol consumption and smoking was also solicited. An interviewer administered the questionnaire to the few subjects who were unable to read it for any reason.

DATA ANALYSIS

Sign severity was evaluated on the UPDRS 5-point scale (none, slight, mild, marked, and severe). Outcomes were assessed in two ways. The first defined parkinsonism using the four cardinal signs: resting tremor, rigidity, bradykinesia, and postural reflex impairment. Subjects were considered to have parkinsonism if they had two or more of these signs, or

if they had one sign and were on anti-parkinsonian medication. These criteria have been used in other studies of parkinsonism prevalence.[37,38] Parkinsonism cases were further categorized by severity of the signs leading to their diagnosis (e.g., slight or greater, mild or greater). Prevalence ratio analyses were performed for these measures using a generalized linear model with binomial distribution and a log link function.[41]

The second method for assessing outcome used the following continuous measures: number of finger taps, number of hand movements, number of rapid alternating movements, duration of maintaining balance while standing (feet tandem, eyes open and closed; feet semi-tandem, eyes open and closed; feet side by side, eyes open and closed), time to walk a fixed distance, and steps to walk a fixed distance. Analyses were performed using multiple regression adjusting for age. In all analyses, a 5% two-sided level of significance was used.

RESULTS

The 323 subjects in this study had a mean age of 69.4 years (range: 49-96) (Table 1). Non-exposed subjects tended to be younger than exposed subjects, with mean ages of 65.8 and 70.2 years, respectively. As would be expected, exposed subjects with the least years of exposure were younger on average (69.2 years) than those with the most years of exposure (73.3 years). All subjects were male; 307 (95.0%) were non-Hispanic Caucasian. A large proportion (42.7%) reported consuming less than one alcoholic drink per month, and a similar proportion (45.8%) reported being current alcohol drinkers. These proportions were similar across exposure subgroups. Although one third of the subjects had never smoked cigarettes, almost half reported 10 or more pack-years of smoking, and 23.8% reported over 35 pack-years. Non-exposed subjects tended to have more pack-years of smoking than exposed subjects. Only 1 subject (0.3%), an orchardist, reported being diagnosed with Parkinson's disease by a physician.

Resting tremor was found in only 7 subjects – 5 (1.5%) with slight tremor and 2 (0.6%) with mild tremor (Table 2). Rigidity was the most commonly observed parkinsonian sign

and the only one presenting at the highest level ("severe"). Almost half of all subjects (n=160 [49.5%]) showed slight rigidity in at least one limb; 87 (26.9%) showed some mild rigidity; 3 (0.9%) showed marked rigidity; and 2 (0.6%) showed severe rigidity. Slight and mild bradykinesia were found in 48 (14.9%) and 8 (2.5%) subjects, respectively. Only slight postural reflex impairment was observed, although it was found in 41 (12.7%) subjects.

There were 67 (20.7%) subjects with slight or greater parkinsonism and 5 (1.5%) with mild or greater parkinsonism (Table 3). We observed no subjects with marked or severe parkinsonism. Of the 67 subjects with slight or greater parkinsonism, 66 had two or more parkinsonian signs; the remaining subject, with one sign, had physician-diagnosed Parkinson's disease and was on anti-parkinsonian medication. Prevalence of parkinsonism increased with age, from 6.8% among subjects younger than 65 years of age to 66.7% for those 85 years of age or older. The last age group ($\geq$ 85 years) contained only 6 subjects; thus, the prevalence estimate for this stratum was unstable. However, the trend of increasing prevalence with age was evident from the other age categories.

There was a higher prevalence of slight or greater parkinsonism among current smokers (31.0%) than among non or former smokers (19.5%) (Table 4). Prevalence was similar among those who had ever smoked at least 100 cigarettes (19.8%) and those who had not (21.3%). Prevalence showed no apparent trend with pack-years of smoking.

As expected, the continuous measures of neurological function showed inverse relations with age (Table 5). Between the youngest and oldest groups, the number of finger taps decreased from 30.4 to 27.6; number of hand movements (opening and closing of hands) went from 27.3 to 23.2; and number of rapid alternating movements (pronation/supination of hands) declined from 16.8 to 13.7. The duration (up to 10 seconds) which the subject was able to stand without swaying or otherwise losing his balance showed the same pattern of decrease from youngest to oldest: 9.6 to 3.0 seconds with feet tandem and eyes open; 7.0 to 0.6 seconds with feet tandem and eyes closed; 10.0 to 8.0 seconds with feet semi-tandem and eyes open; 10.0 to 5.6 seconds with feet

semi-tandem and eyes closed. All subjects were able to maintain their balance for the entire test only when standing with their feet side by side (with eyes both open and closed). The time and number of steps needed to walk ten feet, turn around, and return increased from youngest to oldest, with the increase in time from 8.5 to 13.0 seconds and in steps from 9.8 to 15.2. All of the observed trends were statistically significant.

We found no significant associations of parkinsonism with any of the smoking measures examined (Table 6). Current smokers had an age-adjusted prevalence ratio (PR) of 1.6 (95% confidence interval: 0.9, 2.7), whereas ever smokers had an age–adjusted PR of 0.9 (0.6, 1.4). Parkinsonism also was unrelated to pack-years of smoking, with PRs of 0.9-1.0 across pack-year categories.

Because of the unusually high age-specific prevalences of rigidity that we observed, the above analyses were repeated using a minimum cutoff of mild for rigidity severity (i.e., requiring that rigidity be mild or greater when assessing slight or greater parkinsonism). This reduced the prevalence of slight or greater parkinsonism from 20.7% to 13.0%. Prevalence ratios were lower for ever vs. never smokers (PR=0.6 [95% CI=0.3, 1.0]) and among pack-year groups (0.3 [0.1, 1.2], 0.7 [0.4, 1.4], 0.8 [0.4, 1.5] for 0.1-9.9, 10.0-34.9, and $\geq$ 35 pack-years, respectively); however, confidence intervals were wide and the estimates were not significant.

DISCUSSION

We found parkinsonism, as defined in this study, to be very common among persons aged 65 and older in this cohort. We found only one case of physician-diagnosed Parkinson's disease. We also observed a strong relationship between age and risk of parkinsonism.

Although the subjects in this study do not comprise a random population sample, they all belong to a cohort that was established in the early 1970s, before most of them were likely to be exhibiting signs of parkinsonism. However, these subjects were originally recruited on the basis of their occupational pesticide exposure and, thus, may have risk

factors for parkinsonism that are not shared (or shared to the same degree) by the general population. Subsequent analyses will address the association between parkinsonism and pesticide exposure.

Several investigators have estimated the prevalence of parkinsonism in different populations. These estimates vary widely between studies. A door-to-door survey in Sicily[38] found prevalence of parkinsonism among men ranging from 0.8% to 3.7% in the 60-69 and 80-99 year age groups, respectively. Population-based studies comprising the EUROPARKINSON project (in France, Italy, the Netherlands, and Spain) reported parkinsonism prevalence among men varying from 0.7-1.5% in the 65-74 age group to 4.0-7.5% in the 85-99 age group.[37] Bennett et al[36] reported a high prevalence of parkinsonism among elderly residents of Boston, with estimates from 14.7% to 53.0% for age groups 65-74 and ≥ 85, respectively. These studies have also observed the consistently increasing prevalence of parkinsonism with age.

The large discrepancy between studies is likely due in part to differences in study population demographics. However, given the lack of a diagnostic "gold standard," it is probably due also to differences in interpretation and quantification of parkinsonian signs. The two European studies[37,38] used the same diagnostic criteria as our study. The Boston study[36] used somewhat different criteria, defining four parkinsonian sign categories – bradykinesia, gait disturbance, rigidity, and tremor – and defining parkinsonism as the presence of two or more categories, with each containing two or more parkinsonian signs. However, these studies did not indicate at what severity a sign was considered "present." Cases in our study presented with only slight or mild signs of parkinsonism. Small differences in the assessment of such subtle parkinsonian signs among studies could substantially affect prevalence estimates.

We found no inverse association between cigarette smoking and risk of parkinsonism, although ever smokers had a lower prevalence when rigidity was defined as mild in severity. An apparent protective effect of smoking on risk of Parkinson's disease has been observed consistently in other studies.[42] Smokers in our study had comparable pack-

years of smoking as smokers in other studies reporting a protective effect against Parkinson's disease.[43-45] However, to the best of our knowledge, smoking has not been examined in relation to other forms of parkinsonism. It may be that tobacco smoke has less pronounced effects on slight parkinsonian signs. Alternatively, smoking may be less or non-protective against the factors contributing to parkinsonism in this population, most of which was comprised of persons occupationally exposed to pesticides. Possible misclassification of parkinsonism status due to the mildness of signs observed in most cases may also have masked an association with smoking.

The age-specific prevalences of rigidity in this group were high compared to those reported elsewhere.[36,46] This might represent overascertainment by the nurse performing the neurological examination. Examinations have been conducted by neurologists in most other population prevalence studies.[36-39] However, while using a higher severity cutoff for rigidity decreased the estimated prevalence of parkinsonism, it did not alter trends with age or smoking.

There is a possible volunteer bias given that only 323 of the 482 living original cohort members who were located participated in this study. This can be explained in part by the fact that the original study covered a wide geographic area and some subjects also had moved away in the intervening decades; many subjects lived an hour or more away by car from the testing center. It is likely that most of these subjects did not participate primarily because of the inconvenience. However, it is possible that this also resulted in the participation of healthier, more mobile persons, and thus may have reduced the prevalence of more severe parkinsonism observed in our sample.

Alternatively, it could be argued that participants comprised a less healthy cross-section of the full surviving cohort since healthier individuals might still have been working and, therefore, have had less time to come in for testing. Although much of this cohort was still employed, there are several facts arguing against this interpretation. First, most of the testing occurred during relatively slow months for orchardists. Second, testing times included weekends in order to facilitate participation by working persons. Third, the

duration of testing could be shortened for those persons unable to commit to the full 3-4 hour assessment for any reason.

In conclusion, we observed a high prevalence of slight or greater parkinsonism in this elderly male cohort of rural residents. Prevalence increased with age but appeared unrelated to smoking history. The lack of a protective effect of smoking may be due to the mild nature of the parkinsonism observed here or to the particular factors contributing to parkinsonism in this population. Further study of the factors leading to parkinsonism will focus on pesticide exposures that have been of interest in investigations of idiopathic Parkinson's disease.[6-8,10,12,14,15,17,21-23,25,27,47,48]

Table 1. Selected demographics of subjects

Characteristic	n (%)				
	Total	Years of occupational pesticide exposure			
		0	1-30	31-50	> 50
	N=323	N=41	N=87	N=102	N=80
Age					
49-65	88 (27.2)	19 (46.3)	24 (27.6)	30 (29.4)	10 (12.5)
65-74	146 (45.2)	14 (34.1)	43 (49.4)	47 (46.1)	36 (45.0)
75-84	81 (25.1)	8 (19.5)	20 (23.0)	24 (23.5)	29 (36.3)
85-96	6 (1.9)	0 (0.0)	0 (0.0)	1 (1.0)	5 (6.3)
Race					
Caucasian, non-Hispanic	307 (95.0)	40 (97.6)	83 (95.4)	99 (97.1)	74 (92.5)
African American	1 (0.3)	0 (0.0)	0 (0.0)	1 (1.0)	0 (0.0)
Native American	6 (1.9)	1 (2.4)	2 (2.3)	0 (0.0)	3 (3.8)
Asian/Pacific Islander	4 (1.2)	0 (0.0)	1 (1.1)	2 (2.0)	1 (1.3)
Alcohol consumption					
Never or less than 1 drink/ month	138 (42.7)	14 (34.1)	39 (44.8)	44 (43.1)	35 (43.8)
Stopped > 6 months ago	34 (10.5)	7 (17.1)	11 (12.6)	5 (4.9)	8 (10.0)
Current drinker	148 (45.8)	20 (48.8)	37 (42.5)	52 (51.0)	37 (46.3)
Current cigarette smoker	29 (9.0)	5 (12.2)	7 (8.0)	8 (7.8)	8 (10.0)
Pack-years of cigarette smoking					
0	108 (33.4)	9 (22.0)	29 (33.3)	39 (38.2)	28 (35.0)
0.1 – 9.9	52 (16.1)	4 (9.8)	13 (14.9)	21 (20.6)	14 (17.5)
10.0 – 34.9	76 (23.5)	15 (36.6)	22 (25.3)	17 (16.7)	18 (22.5)
≥ 35.0	77 (23.8)	11 (26.8)	22 (25.3)	22 (21.6)	19 (23.8)
Physician-diagnosed Parkinson's disease (self-reported)	1 (0.3)	0 (0.0)	1 (1.1)	0 (0.0)	0 (0.0)

Table 2. Prevalence by quintile of selected parkinsonian signs

	n (%)				
	Sign severity				
Sign	**None**	**Slight**	**Mild**	**Marked**	**Severe**
Resting tremor	315 (97.5)	5 (1.5)	2 (0.6)	0 (0.0)	0 (0.0)
Rigidity (in any limb)	163 (50.5)	160 (49.5)	87 (26.9)	3 (0.9)	2 (0.6)
Left arm	119 (36.8)	135 (41.8)	63 (19.5)	1 (0.3)	2 (0.6)
Right arm	114 (35.3)	132 (40.9)	72 (22.3)	3 (0.9)	1 (0.3)
Left leg	139 (43.0)	110 (34.1)	64 (19.8)	1 (0.3)	2 (0.6)
Right Leg	136 (42.1)	104 (32.2)	73 (22.6)	1 (0.3)	2 (0.6)
Bradykinesia	265 (82.0)	48 (14.9)	8 (2.5)	0 (0.0)	0 (0.0)
Postural reflex impairment	275 (85.1)	41 (12.7)	0 (0.0)	0 (0.0)	0 (0.0)
Postural tremor	302 (93.5)	16 (5.0)	4 (1.2)	0 (0.0)	0 (0.0)
Action tremor	271 (83.9)	49 (15.2)	2 (0.6)	0 (0.0)	0 (0.0)
Speech impairment	267 (82.7)	54 (16.7)	1 (0.3)	0 (0.0)	0 (0.0)
Hypomimia	267 (82.7)	53 (16.4)	2 (0.6)	0 (0.0)	0 (0.0)
Finger taps	177 (54.8)	122 (37.8)	21 (6.5)	1 (0.3)	0 (0.0)
Hand movements	178 (55.1)	120 (37.2)	23 (7.1)	1 (0.3)	0 (0.0)
Rapid alternating movements	174 (53.9)	111 (34.4)	33 (10.2)	4 (1.2)	0 (0.0)
Rising from a chair	289 (89.5)	22 (6.8)	8 (2.5)	1 (0.3)	0 (0.0)
Gait (walk 10 ft, return, sit in chair)	275 (85.1)	40 (12.4)	5 (1.5)	1 (0.3)	0 (0.0)
Overall judgment of bradykinesia	265 (82.0)	48 (14.9)	8 (2.5)	0 (0.0)	0 (0.0)

Table 3. Parkinsonism prevalence by age

	n (%)		
	Parkinsonism by sign severity		
Age	**None**	**≥ Slight**	**≥ Mild**
49-65	81 (92.0)	6 (6.8)	0 (0.0)
65-74	119 (81.5)	27 (18.5)	2 (1.4)
75-84	52 (64.2)	29 (35.8)	3 (3.7)
85-96	2 (33.3)	4 (66.7)	0 (0.0)
Total	255 (78.9)	67 (20.7)	5 (1.5)

Table 4. Parkinsonism by smoking status

		n (%)		
		Parkinsonism by sign severity		
Smoking status	**Total**	**None**	**≥ Slight**	**≥ Mild**
Current smoker				
Yes	29	20 (69.0)	9 (31.0)	0 (0.0)
No	292	235 (80.5)	57 (19.5)	5 (1.7)
Ever smoked ≥ 100 cigarettes				
Yes	212	170 (80.2)	42 (19.8)	2 (0.9)
No	108	85 (78.7)	23 (21.3)	3 (2.8)
Pack-years				
0.0	108	85 (78.7)	23 (21.3)	3 (2.8)
0.1-9.9	52	43 (82.7)	9 (17.3)	0 (0.0)
10.0-34.9	76	60 (78.9)	16 (21.1)	1 (1.3)
≥ 35	77	60 (77.9)	17 (22.1)	1 (1.3)

Table 5. Selected continuous neurological signs by age

Sign	Total		49-65		65-74		75-84		85-96	
	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)
Finger taps (# in 10 secs)	322	28.7 (4.8)	87	30.4 (3.9)	146	28.5 (4.8)	81	27.4 (5.0)	6	27.6 (5.4)*
Left	320	28.6 (4.5)	87	29.9 (4.0)	145	28.5 (4.5)	81	27.3 (4.8)	5	29.2 (2.6)*
Right	320	29.0 (5.2)	87	30.9 (4.1)	144	28.7 (5.2)	81	27.6 (5.7)	6	28.0 (5.5)*
Hand movements (# in 10 secs)	322	25.3 (4.4)	87	27.3 (3.8)	146	25.0 (4.5)	81	23.9 (4.2)	6	23.2 (5.9)*
Left	321	25.4 (4.3)	87	27.1 (3.7)	145	25.1 (4.4)	81	24.1 (4.2)	6	24.5 (5.6)*
Right	322	25.2 (4.8)	87	27.4 (4.1)	146	24.9 (4.8)	81	23.6 (4.3)	6	21.8 (6.8)*
Rapid alternating movements (# in 10 secs)	322	15.3 (3.1)	87	16.8 (2.8)	146	15.1 (3.0)	81	14.2 (2.9)	6	13.7 (3.1)*
Left	319	15.2 (3.1)	87	16.5 (2.6)	143	15.0 (3.0)	81	14.3 (3.1)	6	13.7 (3.9)*
Right	322	15.4 (3.3)	87	17.1 (3.2)	146	15.1 (3.1)	81	14.1 (2.9)	6	3.7 (2.4)*
Timed stand (0-10 secs)										
Tandem, eyes open	313	7.8 (3.6)	84	9.6 (1.2)	145	7.8 (3.5)	77	6.2 (4.2)	5	3.0 (4.5)*
Tandem, eyes closed	312	4.5 (4.0)	83	7.0 (3.7)	145	4.3 (3.7)	77	2.6 (3.3)	5	0.6 (1.3)*
Semi-tandem, eyes open	316	9.8 (1.4)	86	10.0 (0.0)	145	9.8 (1.4)	78	9.5 (1.7)	5	8.0 (3.5)*
Semi-tandem, eyes closed	315	9.3 (2.3)	86	10.0 (0.0)	144	9.3 (2.2)	78	8.7 (2.9)	5	5.6 (5.2)*
Side by side, eyes open	317	10.0 (0.0)	86	10.0 (0.0)	146	10.0 (0.0)	78	10.0 (0.0)	5	10.0 (0.0)
Side by side, eyes closed	316	10.0 (0.0)	86	10.0 (0.0)	145	10.0 (0.0)	78	10.0 (0.0)	5	10.0 (0.0)
Gait (walk 10 ft, turn, return)										
Time	318	9.1 (1.6)	86	8.5 (1.3)	144	9.1 (1.6)	81	9.5 (1.5)	5	13.0 (2.9)*
Steps	318	10.6 (1.7)	86	9.8 (1.2)	144	10.6 (1.3)	81	11.1 (1.6)	5	15.2 (5.4)*

* Significant trend with age, $p < 0.001$

Table 6. Parkinsonism prevalence ratios by age and smoking history

		PR (95% CI) [≥ slight parkinsonism]	
Factor	**N**	**Unadjusted**	**Adjusted***
Age			
49-65	88	1.0	N/A
65-74	146	2.7 (1.2, 6.3)	N/A
75-84	81	5.3 (2.3, 12.0)	N/A
85-96	6	9.8 (3.8, 25.5)	N/A
Ever smoker†	320	0.9 (0.6, 1.5)	0.9 (0.6, 1.4)
Current smoker	321	1.6 (0.9, 2.9)	1.6 (0.9, 2.7)
Pack-years			
0.0	108	1.0	1.0
0.1-9.9	52	0.8 (0.4, 1.6)	0.9 (0.5, 1.7)
10.0-34.9	76	1.0 (0.6, 1.7)	0.9 (0.5, 1.5)
≥ 35	76	1.0 (0.6, 1.8)	1.0 (0.6, 1.6)

* Adjusted for age

† Defined as ever smoking 100 or more cigarettes

CHAPTER 2: PARKINSONISM AND OCCUPATIONAL EXPOSURE TO PESTICIDES

ABSTRACT

Objective: To examine the risk of parkinsonism related to lifetime occupational pesticide exposure among a cohort of men, mostly orchardists, in Washington State. *Methods:* All 323 subjects in this study had previously participated in a cohort study of men occupationally exposed to pesticides. Subjects were given a structured neurologic examination and completed a self-administered questionnaire which elicited detailed information on pesticide (insecticide, herbicide, and fungicide) use throughout their working careers, and demographic characteristics. Subjects had a mean age of 69.4 years (range: 49-96, standard deviation: 8.1). There were 269 (83.3%) subjects who reported some occupational pesticide exposure, whereas 41 (12.7%) reported none. Parkinsonism was defined by the presence of two or more of resting tremor, rigidity, bradykinesia, and postural reflex impairment in subjects not on anti-parkinsonian medication, or the presence of one sign if they were on such medication. Parkinson's disease was not studied explicitly because it could not be distinguished from other parkinsonian syndromes. A generalized linear model was used to estimate prevalence ratios for parkinsonism in relation to history of farming, pesticide use, and well water use. *Results:* A prevalence ratio of 2.0 (95% CI: 1.0, 4.2) was observed for subjects in the highest tertile of years of pesticide exposure; the prevalence ratio for the middle tertile was elevated but non-significant. However, we observed no increased risk associated with specific pesticides or pesticide classes, nor with a history of farming or well water use. *Conclusion:* There was an increased risk of parkinsonism associated with long-term occupational exposure to pesticides, but we could not detect any associations between parkinsonism and specific pesticides.

INTRODUCTION

Parkinsonism, which is characterized by rigidity, resting tremor, bradykinesia, and postural reflex impairment, is primarily due to decreased activity of the neurotransmitter dopamine in the nigrostriatal system.[1,2] Parkinsonism has been associated with several chemical exposures, including manganese, carbon disulfide and other organic solvents, and carbon monoxide.[1]

Little is known about the cause of idiopathic Parkinson's disease, which is a distinct disease entity among parkinsonian syndromes, despite a great deal of scientific interest and the passage of nearly two centuries since its first recognition. However, mounting evidence suggests an etiologic role of environmental factors. The first such evidence was the discovery of severe parkinsonism remarkably similar to Parkinson's disease in intravenous drug abusers exposed to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).[3] This condition was caused by degeneration of dopaminergic neurons in the nigrostriatal pathway similar to that found in idiopathic Parkinson's disease, a fact which distinguished it from other parkinsonism-inducing agents. Structural similarity between MPTP and the herbicide paraquat[4] has led to speculation of a possible association between exposure to pesticides and Parkinson's disease.

Several studies have investigated the relation between exposure to rural factors and Parkinson's disease (PD), producing equivocal results. Some studies have reported an increased risk of PD associated with rural residence[6,7,11,16,19,21,28,30,49,50] or farming,[12,16] but others have found no such associations.[15,22,24] The only epidemiologic study we are aware of that has examined parkinsonism, as opposed to Parkinson's disease, found increased risks of extrapyramidal signs among persons with a history of field or landscape work.[13] Consumption of well water has also been associated with risk of PD in some studies[9,19,27,30,49] but not others.[12,15,18,23,24,28,29] Such discrepant findings may be due to differences in study design, subject demographics, or exposure types and levels.

Numerous studies have also examined associations between pesticides and Parkinson's disease, although few specific chemicals or chemical classes have been implicated causally. While most of these studies have observed increased risk associated with pesticides,[6,7,10-12,15-18,23,25] others have been negative.[20,21,27,28,30,49] Conflicting results have also been observed for paraquat and PD.[14,15,18,21]

The present study examined the risk of parkinsonism in relation to lifetime occupational pesticide exposure among a cohort of men, mostly orchardists, in central Washington State. All subjects were participants in an earlier cohort study of men occupationally exposed to pesticides which was begun in the early 1970s by the Washington State Department of Health. Subjects in the present study were given a structured neurological examination and asked to complete a detailed questionnaire covering demographics, lifestyle factors, and lifetime pesticide exposure. Parkinsonism was defined as the presence of two or more of the following signs: bradykinesia, resting tremor, rigidity, and postural reflex impairment. This study did not attempt to distinguish idiopathic Parkinson's disease from other forms of parkinsonism which can be caused by a variety of factors.

METHODS

SUBJECTS

The location and recruitment of subjects have been described elsewhere (see Chapter 1). In brief, all subjects had previously participated in a cohort study carried out by the Washington State Department of Health from 1972 through 1976. Pesticide-exposed subjects in that study consisted primarily of orchardists (n=739, 56.8%), professional pesticide applicators (n=30, 2.3%), pesticide formulation plant workers (n=7, 0.5%), and other farm or agricultural workers (n=6, 0.5%). Non-exposed subjects were frequency-matched to exposed subjects by age, race, and work regimen (i.e., degree of physical

activity). Subjects were male, mostly non-Hispanic Caucasian (95.0%), and aged 18 to 88 at the time they entered that study.

Using information from various sources, including contact information provided by subjects in the original study, we were able to locate 482 surviving members of the original 1,300 subject cohort. They were invited in the summer of 1997 to participate in the current study and 323 (67.0%) accepted. They then underwent a 3-4 hour assessment at a centrally located testing center. The study protocol was approved by the University of Washington and Oregon Health Sciences University Human Subjects Committees, and all participants provided written, informed consent.

EXAMINATION

A neurologist-trained nurse administered an approximately 20 minute structured, uniform neurological examination to all subjects. The presence and severity of motor signs were recorded by the nurse using the United Parkinson's Disease Rating Scale (UPDRS).[40] Signs included postural, action, and resting tremors, rigidity in each limb, postural reflex impairment, bradykinesia, as well as speech impairment, hypomimia, and slowness of: finger taps (repeated tapping of thumb with index finger), hand movements (repeated opening and closing of hands), rapid alternating movements (repeated pronation and supination of hands), rising from a chair, and gait.

PESTICIDE USE ASCERTAINMENT

Each subject was asked to complete a self-administered questionnaire which asked detailed questions about the subject's use of pesticides (insecticides, herbicides, and fungicides) throughout his working career. The subject was asked to provide information on years of farming or occupational pesticide-related employment and, for discrete time periods: crops grown; number of acres of each crop; use (i.e., mixing, loading, or applying) of specific insecticides, herbicides, and fungicides (provided in a comprehensive list appropriate to the region); target crops for each pesticide used; typical

methods of application; and use of protective clothes and equipment. Most of the above information was solicited by calendar decade; use of specific pesticides was solicited by 5-year period as far back as 1960, and open-ended before then. The subject was also asked to report any pesticides not included in the list provided. Demographic information, including age, race, health conditions, use of medications, and history of alcohol consumption and smoking was solicited. An interviewer administered the questionnaire to the few subjects who were unable to read it for any reason.

DATA ANALYSIS

Pesticide exposure was assessed in several ways. These included the dichotomous measures of 1) any history of farm employment; 2) any history of use of insecticides, herbicides, and fungicides, as well as the more specific categories of carbamates, organophosphates, organochlorines, dithiocarbamates, and manganese-containing pesticides (which are also included in the dithiocarbamate category); and 3) any history of use of specific pesticides, such as azinphos methyl, DDT, ferbam, lead arsenate, mancozeb, maneb, methyl parathion, paraquat, TEPP, thiram, zineb, and ziram (most of which are contained in the above chemical classes). We also considered continuous measures of 1) years of exposure to, or use of, the above factors; and 2) number of acre-years using the previously mentioned pesticides (= number of years of use × number of acres of the particular crop(s) to which it was applied). Analyses involving continuous measures of exposure were restricted to subjects reporting some use of the appropriate chemical or chemical class in order to reduce heterogeneity in potential confounding factors (i.e., subjects with the lowest non-zero exposure were used as the reference group). Since pre-1960 pesticide use information was solicited only as yes or no for each pesticide, the first year of use for a pesticide used by a subject before 1960 was assumed to be either the pesticide's year of introduction or the subject's first year of farming, whichever came later. Specific pesticides and pesticide classes were chosen for analyses based on their potential neurotoxicity (e.g., manganese-containing pesticides,

dithiocarbamates which can produce carbon disulfide, paraquat because of its structural similarity to MPTP).

Parkinsonism was defined based on the presence of the four cardinal signs: resting tremor, rigidity, bradykinesia, and postural reflex impairment. A sign was considered present if observed at a level of slight or greater on the UPDRS. Subjects were considered to have parkinsonism if they had two or more of these signs, or if they had one sign and were on anti-parkinsonian medication. These criteria have been used in other studies of parkinsonism.[37,38]

Prevalence ratio analyses were performed on all exposure measures for which there was a sufficient number of parkinsonism cases. Prevalence ratios were estimated from a generalized linear model with binomial distribution and a log link function.[41] Adjustment was made for age and pack-years of cigarette smoking. In all analyses, a 5% two-sided level of significance was used.

RESULTS

The 323 subjects in this study had a mean age of 69.4 years (range: 49-96) (Table 7). Non-exposed subjects tended to be younger than exposed subjects, with mean ages of 65.8 and 70.2 years, respectively. As would be expected, exposed subjects with the least years of exposure were younger on average (69.2 years) than those with the most years of exposure (73.3 years). All subjects were male; 307 (95.0%) were non-Hispanic Caucasian. A large proportion (42.7%) reported consuming less than one alcoholic drink per month, and a similar proportion (45.8%) reported being current alcohol drinkers. These proportions were similar across exposure subgroups. Although one third of the subjects had never smoked cigarettes, almost half reported 10 or more pack-years of smoking, and 23.8% reported over 35 pack-years. Non-exposed subjects tended to have more pack-years of smoking than exposed subjects. Only 1 subject (0.3%), an orchardist,

reported being diagnosed with Parkinson's disease by a physician. There were 67 (20.7%) subjects with signs of parkinsonism.

No significant associations were observed between parkinsonism and ever use of well water, farm employment, or exposure to pesticides when adjusted for age and smoking (Table 8). Significantly reduced prevalence ratios (PR) were observed for unadjusted estimates for the manganese-containing pesticide class (PR: 0.3 [95% confidence interval: 0.1, 0.9]) and the specific manganese-containing fungicide mancozeb (0.3 [0.1, 0.9]). The number of cases with exposure to manganese-containing pesticides (mancozeb and maneb), ferbam, thiram, and zineb was very small, preventing further analyses of these agents.

Analyses by tertiles of years of exposure showed a significantly elevated adjusted prevalence ratio for the highest tertile of general pesticide exposure (2.0 [1.0, 4.2]) (Table 9). The prevalence ratio for the middle tertile of this exposure was elevated but non-significant, and a trend test was also non-significant (p=0.17). The middle tertile of azinphos methyl exposure had a significantly reduced adjusted prevalence ratio (0.5 [0.2, 1.0], p=0.11), although no significant effect was observed in the upper tertile. Significant duration-response relations, as determined by tests of trend, were observed only for crude analyses of exposure to pesticides, insecticides, and lead arsenate. We found no significant associations between parkinsonism and acre-years of any pesticide category (Table 10).

Because owners of small farms may be more likely to apply pesticides themselves and, thus, potentially receive greater exposures than owners of large farms, we repeated the above analyses restricted to farmers whose acreage averaged over time was in the bottom tertile and also those farmers whose acreage averaged over time was in the upper tertile (data not shown). Results were generally similar to those observed for the full cohort. However, we did observe a significantly elevated adjusted prevalence ratio for the middle tertile of years of organochlorine exposure (9.5 [1.3, 69.4]) among farmers with average

acreage in the upper tertile. The highest tertile of organochlorine exposure also showed a substantially increased, but non-significant, prevalence ratio (4.1 [0.5, 33.9]).

DISCUSSION

We found parkinsonism, as defined in this study, to be associated with long-term occupational exposure to pesticides. However, we observed no increased risk of parkinsonism associated with specific pesticides, nor from duration of farm employment or well water use.

Our focus on pesticides in relation to parkinsonism was motivated in part by the frequent observation of higher rates of Parkinson's disease in rural areas. An ecologic study by Barbeau et al.[6] found a strong correlation between region-specific Parkinson's disease prevalence and pesticide use in rural Quebec. Another Canadian study reported increased risk of early-onset Parkinson's disease associated with childhood residence in a rural environment, although it was unable to identify any specific risk factors.[21] Stern et al.[28] implicated a history of rural living, but found no association with pesticides or well water. A case-control study of families with two or more siblings with Parkinson's disease found an excess of rural living and well water use among cases compared to controls, but found no association with farming or herbicide exposure.[30] Other investigators have reported similarly mixed findings concerning rural living or well water use.[7,11,16,19,49]

Other motivating factors were the recognized neurotoxicity of some widely used organophosphate pesticides[51] and the similarity of chemical structure between MPTP and the herbicide paraquat.[4] Reports of parkinsonism induced by chronic exposure to the manganese-containing fungicide maneb[47] and by organophosphate intoxication[52] provided additional justification for our study.

A study of parkinsonism, as opposed to the more specific Parkinson's disease, among kibbutz residents in Israel[13] found increased risks of extrapyramidal signs among subjects with a history of field crop and landscape work. The association was particularly strong for a history of field work with cotton, which typically requires more pesticides than most other crops. Evidence from related research by these authors suggests that these extrapyramidal signs may represent an early stage of Parkinson's disease.[53,54]

Research by other investigators suggests a link between pesticide exposure and idiopathic Parkinson's disease. Similar to our results, Hertzman et al.[15] observed a doubling of risk of Parkinson's disease among men with occupational pesticide exposure – primarily as orchardists – but were unable to associate the increased risk with any specific pesticides. An earlier study by Hertzman et al.[14] found increased risks of Parkinson's disease from working in orchards and handling paraquat. Several authors have reported an increased risk associated with farming.[7,12,16] Butterfield et al.[7] observed elevated risks of Parkinson's disease from exposure to insecticides and herbicides, but not fungicides. A recent study by Gorell et al.[12] had similar findings. Ho et al.[16] also observed positive associations between insecticides and herbicides and Parkinson's disease. A positive exposure-response gradient was found between duration of herbicide/pesticide use and Parkinson's disease risk by Liou et al.[18] Semchuk et al.[25] found an increased risk only for herbicide use. A recent large case-control study from Germany[23] is one of the first to identify specific pesticide chemical classes as risk factors for Parkinson's disease. This study found elevated odds ratios for exposure to organochlorines and alkylated phosphates, including an exposure-response gradient for the latter.

Despite the chemical similarity of paraquat and MPTP, studies of the association between paraquat and Parkinson's disease have produced mixed results. Hertzman et al.[14] observed a significant positive association among a small number of subjects exposed to this chemical in a Canadian case-control study. However, a later study by these authors[15] failed to repeat this finding. Rajput et al.,[21] in a Canadian study of early-onset Parkinson's disease, also found no association. The strongest evidence comes from a

Taiwanese study by Liou et al.[18] which reported an exposure-response relation between years of paraquat use and risk of Parkinson's disease. The cause of these variable results is unclear, but may relate to differences in study populations or to recall bias in these case-control studies.

Findings of an association between well water use and Parkinson's disease are also inconsistent, with some investigators reporting an increased risk,[9,19,27,30,49] but others reporting none.[12,15,18,23,24,28] Some of the discrepancy between studies might be explained by differences in the depth, distance from farms, or duration of use of the wells. Our study found no association between parkinsonism and either history of well water use or duration of that use, despite considering various well-to-farm distances of up to one mile.

Our study has several potential biases and limitations. There is the possibility of a recall bias in which parkinsonism cases were more likely to remember or overreport pesticide use than non-cases. However, this is unlikely for two reasons. The first is that an increased risk of parkinsonism was observed only for general pesticide use and not for any specific pesticides. Furthermore, most of our subjects with parkinsonism showed only slight parkinsonian signs (with only one case of physician-diagnosed Parkinson's disease) and did not appear substantially impaired by it. Recall error by our subjects was more likely to be non-differential, given the large number of pesticides reported and the complex temporal pattern of their use. Also, subjects were not informed of the study hypotheses.

Selection bias is unlikely in this study. Although our subjects do not comprise a random population sample, they all belong to a cohort that was established in the early 1970s, before most of them were likely to be exhibiting signs of parkinsonism. The fact that only 323 of the 482 original cohort members agreed to participate in this study can be explained in part by the fact that the original study covered a wide geographic area and some subjects had moved away in the intervening decades. Many subjects lived an hour or more away by car from the testing center. It is likely that most of these subjects did not

participate primarily because of the inconvenience. However, it is possible that this resulted in the participation of healthier, more mobile persons or, alternatively, less healthy persons who were no longer working. In either case, bias would only be introduced if probability of participation was further influenced by historical pesticide use, which seems unlikely.

Given the large number of exposures investigated, spurious associations due to multiple testing are a possibility. The association between parkinsonism and long-term use of pesticides was only marginally significant. A similarly elevated, non-significant, risk was also observed for medium-term use of pesticides, although a trend test did not demonstrate a significant exposure-response relation. In addition, elevated, though non-significant, risks were associated with long-term use of insecticides and herbicides.

There are several possible explanations why we found an increased risk of parkinsonism for general pesticide use but not for specific pesticides. One is that, as the historical exposure probing of a subject goes from general to specific, the subject may become more prone to error in recalling the details (i.e., names, timing, duration, frequency) of that exposure. Such exposure misclassification is likely to be non-differential with respect to parkinsonism, potentially masking certain associations. We tried to minimize this problem by providing subjects with a comprehensive list of pesticides which they were likely to have used, including various common trade names for each pesticide, in order to facilitate recall. We also used a structured questionnaire which attempted to create a framework for the subject by soliciting information on general context before soliciting information on specifics. Another possibility is that we did not consider the appropriate pesticides despite carefully selecting the most likely candidates. It is also possible that we lacked the statistical power to identify associations with individual pesticides. Although some use of pesticides was reported by most subjects, use of certain pesticides was reported by relatively few. For instance, use of manganese-containing pesticides was reported by only 51 persons; maneb by only 12. Dithiocarbamate use was reported by

less than one third of the cohort. Small numbers for certain pesticides prevented some analyses from being performed altogether.

In conclusion, we observed an increased risk of parkinsonism associated with long-term occupational exposure to pesticides, but were unable to demonstrate any associations with specific pesticides. Follow-up studies in which pesticide use information is collected around the time of use may help to resolve such ambiguity in the future.

Table 7. Selected demographics of subjects

Characteristic	n (%)				
	Total	Years of occupational pesticide exposure			
		0	1-30	31-50	> 50
	N=323	N=41	N=87	N=102	N=80
Age					
49-65	88 (27.2)	19 (46.3)	24 (27.6)	30 (29.4)	10 (12.5)
65-74	146 (45.2)	14 (34.1)	43 (49.4)	47 (46.1)	36 (45.0)
75-84	81 (25.1)	8 (19.5)	20 (23.0)	24 (23.5)	29 (36.3)
85-96	6 (1.9)	0 (0.0)	0 (0.0)	1 (1.0)	5 (6.3)
Race					
Caucasian, non-Hispanic	307 (95.0)	40 (97.6)	83 (95.4)	99 (97.1)	74 (92.5)
African American	1 (0.3)	0 (0.0)	0 (0.0)	1 (1.0)	0 (0.0)
Native American	6 (1.9)	1 (2.4)	2 (2.3)	0 (0.0)	3 (3.8)
Asian/Pacific Islander	4 (1.2)	0 (0.0)	1 (1.1)	2 (2.0)	1 (1.3)
Alcohol consumption					
Never or less than 1 drink/ month	138 (42.7)	14 (34.1)	39 (44.8)	44 (43.1)	35 (43.8)
Stopped > 6 months ago	34 (10.5)	7 (17.1)	11 (12.6)	5 (4.9)	8 (10.0)
Current drinker	148 (45.8)	20 (48.8)	37 (42.5)	52 (51.0)	37 (46.3)
Current cigarette smoker	29 (9.0)	5 (12.2)	7 (8.0)	8 (7.8)	8 (10.0)
Pack-years of cigarette smoking					
0	108 (33.4)	9 (22.0)	29 (33.3)	39 (38.2)	28 (35.0)
0.1 – 9.9	52 (16.1)	4 (9.8)	13 (14.9)	21 (20.6)	14 (17.5)
10.0 – 34.9	76 (23.5)	15 (36.6)	22 (25.3)	17 (16.7)	18 (22.5)
≥ 35.0	77 (23.8)	11 (26.8)	22 (25.3)	22 (21.6)	19 (23.8)
Physician-diagnosed Parkinson's disease (self-reported)	1 (0.3)	0 (0.0)	1 (1.1)	0 (0.0)	0 (0.0)

Table 8. Parkinsonism prevalence ratios (ever/never) by pesticide-related exposure

	n (%) cases		PR (95% CI) for parkinsonism	
Exposure	**Exposed**	**Non-exposed**	**Unadjusted**	**Adjusted***
Well water use	32 (19.2)	18 (19.1)	1.0 (0.6, 1.7)	0.9 (0.6, 1.5)
Farm employment	57 (21.3)	8 (19.0)	1.1 (0.6, 2.1)	1.0 (0.5, 1.8)
Any pesticides	65 (20.8)	2 (20.0)	1.0 (0.3, 3.7)	1.0 (0.2, 6.1)
Any insecticides	51 (21.3)	14 (19.4)	1.1 (0.6, 1.8)	0.9 (0.6, 1.5)
Any herbicides	39 (20.2)	26 (22.0)	0.9 (0.6, 1.4)	0.9 (0.6, 1.3)
Any fungicides	35 (19.0)	28 (22.8)	0.8 (0.5, 1.3)	0.8 (0.6, 1.3)
Any carbamates	31 (19.1)	34 (23.6)	0.8 (0.5, 1.2)	0.8 (0.6, 1.3)
Any organophosphates	48 (21.0)	17 (21.0)	1.0 (0.6, 1.6)	0.9 (0.6, 1.4)
Any organochlorines	45 (20.4)	20 (23.0)	0.9 (0.6, 1.4)	0.8 (0.5, 1.3)
Any dithiocarbamates	19 (17.3)	44 (22.4)	0.8 (0.5, 1.2)	0.8 (0.5, 1.3)
Any manganese-containing	4 (7.8)	58 (23.3)	**0.3 (0.1, 0.9)**	0.4 (0.2, 1.1)
Azinphos methyl	24 (17.5)	24 (21.1)	0.8 (0.5, 1.4)	0.8 (0.5, 1.3)
DDT	40 (20.6)	25 (21.9)	0.9 (0.6, 1.5)	0.8 (0.5, 1.2)
Ferbam	2 (16.7)	59 (20.8)	0.8 (0.2, 2.9)	0.9 (0.3, 3.0)
Lead arsenate	24 (24.5)	28 (18.4)	1.3 (0.8, 2.1)	1.0 (0.6, 1.6)
Mancozeb	3 (6.8)	58 (22.8)	**0.3 (0.1, 0.9)**	0.4 (0.1, 1.2)
Maneb	1 (8.3)	61 (21.4)	0.4 (0.1, 2.6)	0.4 (0.1, 2.6)
Methyl parathion	28 (18.1)	36 (24.0)	0.7 (0.5, 1.2)	0.7 (0.5, 1.1)
Paraquat	20 (17.5)	29 (21.0)	0.8 (0.5, 1.4)	0.8 (0.5, 1.3)
TEPP	12 (18.8)	35 (20.1)	0.9 (0.5, 1.7)	0.8 (0.5, 1.5)
Thiram	2 (16.7)	60 (20.9)	0.8 (0.2, 2.9)	0.8 (0.2, 2.8)
Zineb	1 (20.0)	61 (20.7)	1.0 (0.2, 5.6)	1.2 (0.2, 6.0)
Ziram	19 (18.8)	43 (21.3)	0.9 (0.5, 1.4)	0.9 (0.6, 1.5)

* Adjusted for age and pack-years of smoking

Table 9. Parkinsonism prevalence ratios by tertiles of years of pesticide-related exposure

	PR (95% CI) of parkinsonism[£]				
		Unadjusted		Adjusted[*]	
Factor	**Tertile 1**	**Tertile 2**	**Tertile 3**	**Tertile 2**	**Tertile 3**
Well water use	1.0	0.7 (0.3, 1.7)	1.3 (0.6, 2.7)	0.7 (0.3, 1.5)	1.0 (0.5, 2.1)
Farm employment	1.0	1.1 (0.6, 1.9)	1.3 (0.7, 2.3)	1.1 (0.6, 1.9)	1.0 (0.6, 1.7)
Any pesticides	1.0	1.8 (0.9, 3.8)	**2.5 (1.3, 5.2)**[†]	1.9 (0.9, 4.0)	**2.0 (1.0, 4.2)**
Any insecticides	1.0	1.2 (0.6, 2.5)	**2.0 (1.0, 3.7)**[†]	1.2 (0.6, 2.4)	1.7 (0.9, 3.3)
Any herbicides	1.0	0.7 (0.3, 1.6)	1.5 (0.7, 3.0)	0.7 (0.3, 1.5)	1.7 (0.9, 3.2)
Any fungicides	1.0	0.7 (0.3, 1.7)	1.2 (0.6, 2.4)	0.7 (0.3, 1.4)	0.7 (0.4, 1.5)
Any carbamates	1.0	0.5 (0.2, 1.2)	**0.5 (0.2, 1.0)**	0.5 (0.2, 1.2)	0.6 (0.3, 1.2)
Any organophosphates	1.0	1.2 (0.7, 2.2)	0.9 (0.4, 1.7)	1.3 (0.7, 2.3)	0.8 (0.4, 1.5)
Any organochlorines	1.0	1.7 (0.9, 3.2)	0.8 (0.4, 1.7)	1.3 (0.7, 2.4)	0.7 (0.4, 1.4)
Any dithiocarbamates	1.0	0.7 (0.3, 2.0)	0.7 (0.2, 1.9)	0.6 (0.2, 1.6)	0.6 (0.2, 1.7)
Azinphos methyl	1.0	0.4 (0.2, 1.1)	0.4 (0.2, 1.1)	**0.5 (0.2, 1.0)**	0.6 (0.3, 1.4)
DDT	1.0	0.7 (0.3, 1.6)	1.6 (0.8, 3.2)	0.6 (0.3, 1.3)	1.3 (0.7, 2.6)
Lead arsenate	1.0	1.8 (0.6, 5.6)	**3.6 (1.3, 9.8)**[†]	1.1 (0.4, 2.8)	1.4 (0.6, 3.4)
Methyl parathion	1.0	1.0 (0.4, 2.3)	1.2 (0.5, 2.7)	1.0 (0.4, 2.4)	1.2 (0.5, 2.8)
Paraquat	1.0	0.5 (0.2, 1.7)	1.2 (0.5, 3.2)	0.4 (0.1, 1.4)	0.9 (0.4, 2.4)
TEPP	1.0	1.8 (0.4, 8.8)	2.6 (0.6, 11.4)	1.4 (0.4, 5.2)	2.0 (0.6, 6.9)
Ziram	1.0	0.8 (0.3, 2.2)	0.7 (0.3, 1.9)	0.6 (0.2, 1.6)	0.6 (0.2, 1.7)

[*] Adjusted for age and pack-years of smoking

[†] Test of trend for tertiles 1 through 3 significant at $p < 0.05$

[£] Restricted to subjects with the particular exposure;

N of tertiles 1, 2, and 3, respectively – Well water use: 53, 58, 56; Farm employment: 89, 99, 80; Pesticides: 79, 83, 76; Insecticides: 75, 80, 80; Herbicides: 60, 73, 51; Fungicides: 57, 63, 57; Carbamates: 58, 40, 59; Organophosphates: 71, 87, 70; Organochlorines: 75, 67, 76; Dithiocarbamates: 39, 33, 36; Azinphos methyl: 43, 48, 41; DDT: 47, 81, 59; Lead arsenate: 33, 32, 30; Methyl parathion: 46, 54, 50; Paraquat: 33, 42, 36; TEPP: 19, 21, 22; Ziram: 37, 29, 33;

Range of values (in years) for tertiles 1, 2, and 3, respectively – Well water use: 1-25, 26-60, 61-89; Farm employment: 1-32, 33-50, 51-80; Pesticides: 1-33, 34-51, 52-81; Insecticides: 1-31, 32-48, 49-76; Herbicides: 1-17, 18-27, 28-55; Fungicides: 1-23, 24-37, 38-73; Carbamates: 1-15, 16-25, 26-41; Organophosphates: 1-25, 26-37, 38-51; Organochlorines: 1-19, 20-31, 32-55; Dithiocarbamates: 1-12, 13-22, 23-57; Azinphos methyl: 1-15, 16-25, 26-40; DDT: 1-13, 14-20, 21-51; Lead arsenate: 1-20, 21-27, 28-44; Methyl parathion: 1-10, 11-20, 21-45; Paraquat: 1-10, 11-20, 21-39; TEPP: 1-7, 8-14, 15-51; Ziram: 1-12, 13-22, 23-57

Table 10. Parkinsonism prevalence ratios by tertiles of acre-years of pesticide-related exposure

	PR (95% CI) of parkinsonism[£]				
		Unadjusted		Adjusted[*]	
Factor	**Tertile 1**	**Tertile 2**	**Tertile 3**	**Tertile 2**	**Tertile 3**
Farm employment	1.0	1.3 (0.7, 2.4)	1.4 (0.8, 2.7)	1.0 (0.5, 1.8)	1.3 (0.7, 2.4)
Any pesticides	1.0	1.1 (0.6, 2.2)	1.4 (0.8, 2.7)	0.9 (0.5, 1.8)	1.5 (0.8, 2.8)
Any insecticides	1.0	0.7 (0.3, 1.4)	1.2 (0.7, 2.1)	0.6 (0.3, 1.2)	1.2 (0.7, 2.1)
Any herbicides	1.0	0.5 (0.2, 1.3)	0.8 (0.4, 1.8)	0.5 (0.2, 1.2)	0.8 (0.4, 1.7)
Any fungicides	1.0	0.9 (0.4, 2.0)	0.8 (0.4, 1.9)	0.8 (0.4, 1.7)	0.8 (0.4, 1.7)
Any carbamates	1.0	0.5 (0.2, 1.3)	0.9 (0.4, 2.0)	0.6 (0.3, 1.4)	1.0 (0.5, 2.0)
Any organophosphates	1.0	0.7 (0.4, 1.3)	0.9 (0.5, 1.7)	0.7 (0.4, 1.2)	0.9 (0.5, 1.6)
Any organochlorines	1.0	0.8 (0.4, 1.7)	1.2 (0.6, 2.3)	0.8 (0.4, 1.5)	1.1 (0.6, 2.0)
Any dithiocarbamates	1.0	1.1 (0.3, 3.3)	0.8 (0.2, 2.8)	1.3 (0.5, 3.7)	1.4 (0.5, 4.5)
Azinphos methyl	1.0	1.0 (0.4, 2.4)	0.7 (0.3, 1.9)	1.2 (0.5, 2.8)	0.9 (0.4, 2.2)
DDT	1.0	1.0 (0.4, 2.3)	1.7 (0.8, 3.5)	0.9 (0.4, 2.1)	1.6 (0.8, 3.2)
Lead arsenate	1.0	1.6 (0.6, 4.1)	1.5 (0.6, 4.0)	1.4 (0.6, 3.3)	1.3 (0.5, 3.1)
Methyl parathion	1.0	1.3 (0.5, 3.1)	1.1 (0.5, 2.9)	1.1 (0.5, 2.7)	1.3 (0.5, 3.0)
Paraquat	1.0	0.4 (0.1, 1.5)	0.8 (0.3, 2.2)	0.4 (0.1, 1.5)	0.7 (0.3, 1.9)
TEPP	1.0	1.8 (0.4, 8.7)	2.4 (0.5, 10.7)	1.3 (0.4, 4.9)	2.0 (0.6, 6.9)
Ziram	1.0	0.7 (0.2, 2.1)	0.7 (0.2, 2.1)	0.8 (0.3, 2.2)	1.2 (0.4, 4.1)

[*] Adjusted for age and pack-years of smoking

[£] Restricted to subjects with the particular exposure;

N of tertiles 1, 2, and 3, respectively – Farm employment: 73, 75, 74; Pesticides: 74, 75, 75; Insecticides: 73, 74, 73; Herbicides: 52, 51, 51; Fungicides: 57, 55, 56; Carbamates: 48, 49, 47; Organophosphates: 69, 70, 70; Organochlorines: 65, 65, 65; Dithiocarbamates: 34, 32, 33; Azinphos methyl: 42, 43, 43; DDT: 53, 54, 53; Lead arsenate: 26, 30, 28; Methyl parathion: 46, 47, 46; Paraquat: 32, 33, 33; TEPP: 18, 20, 19; Ziram: 30, 30, 30;

Range of values (in acre-years) for tertiles 1, 2, and 3, respectively – Farm employment: 1-689, 690-1950, 1951-25030; Pesticides: 1-559, 560-1655, 1656-24430; Insecticides: 1-480, 481-1550, 1551-16140; Herbicides: 1-370, 371-1200, 1201-10345; Fungicides: 1-360, 361-1200, 1201-10900; Carbamates: 1-296, 297-880, 881-8630; Organophosphates: 1-455, 456-1367, 1368-20300; Organochlorines: 1-320, 321-1060, 1061-6580; Dithiocarbamates: 1-270, 271-714, 715-6330; Azinphos methyl: 1-280, 281-800, 801-8580; DDT: 1-170, 171-650, 651-5600; Lead arsenate: 1-170, 171-510, 511-4525; Methyl parathion: 1-240, 241-700, 701-5120; Paraquat: 1-250, 251-682, 683-9950; TEPP: 1-140, 141-430, 431-5650; Ziram: 1-275, 276-720, 721-6330

CHAPTER 3: INTERACTIONS BETWEEN PESTICIDE EXPOSURE AND GENETIC POLYMORPHISMS ON PARKINSONISM IN A COHORT OF OCCUPATIONALLY EXPOSED MEN

ABSTRACT

Objective: To examine monoamine oxidase (MAO-B) and NADH dehydrogenase subunit 1 (ND1) polymorphisms, as well as interactions between pesticide exposure and those polymorphisms, in relation to prevalence of parkinsonism. *Methods:* All 323 subjects in this study had previously participated in a cohort study of men occupationally exposed to pesticides, mostly as orchardists. Subjects were given a structured neurologic examination and completed a self-administered questionnaire which solicited detailed information on pesticide (insecticide, herbicide, and fungicide) use throughout their working careers as well as demographic characteristics. MAO-B intron 13 G/A and ND1 nucleotide 4216 (ND1/4216) T/C genetic polymorphisms were also assessed. Parkinsonism was defined by the presence of two or more of resting tremor, rigidity, bradykinesia, and postural reflex impairment in subjects not on anti-parkinsonian medication, or the presence of one sign if they were on such medication. Parkinson's disease was not studied explicitly because it could not be distinguished from other parkinsonian syndromes. A generalized linear model was used to estimate prevalence ratios for parkinsonism in relation to 1) MAO-B and ND1 genotypes, and 2) smoking and pesticide use stratified by genotype. *Results:* We observed no increased risk of parkinsonism associated with any genotype. We also observed no association of parkinsonism with smoking, pesticide exposure, farming, or well water use for any genotype. *Conclusion:* These genetic markers were neither independent risk factors for parkinsonism, nor effect modifiers of the association between pesticides or smoking and parkinsonism.

INTRODUCTION

Parkinsonism, characterized by rigidity, resting tremor, bradykinesia, and postural instability, is primarily due to decreased activity of the neurotransmitter dopamine in the nigrostriatal system. Parkinson's disease is a specific parkinsonian disorder in which the pathologic lesions are limited primarily to the substantia nigra and other pigmented neurons.[1,2] Parkinson's disease has been the focus of most parkinsonism research. However, despite a great deal of prior research of Parkinson's disease, little is known about its etiology.

Genetic susceptibility to Parkinson's disease has received increasing attention in recent years,[5] especially the possibility of gene-environment interactions, in which individuals possessing certain genotypes are at increased risk of disease from certain environmental factors.[31-33] Most studies which have investigated such interactions have focused on the cytochrome P450 enzymes, which play a major role in the detoxification of exogenous compounds. However, polymorphisms in other enzymes may also be important in the etiology of parkinsonism by facilitating either detoxification or bioactivation of exogenous chemicals.

One polymorphism that has been associated with Parkinson's disease (PD) is a single-base substitution in intron 13 of the monoamine oxidase B (MAO-B) gene.[31] This enzyme may play a role in PD etiology via its function in catabolizing dopamine. MAO-B also bioactivates at least one exogenous neurotoxicant, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).[55] Since MPTP is similar in chemical structure to the herbicide paraquat,[4] and since numerous studies have associated rural or agricultural exposures with increased risk of PD,[6,7,10-12,14-19,21,23,25,28,30,49] MAO-B seems a promising candidate for study in relation to pesticide exposure and parkinsonism.

Increasing evidence also suggests an important role for the ND1 subunit of mitochondrial Complex I and for oxidative stress in PD pathogenesis.[56] MPP+, produced via metabolism of MPTP by MAO-B, is selectively taken up by dopaminergic neurons.

There it accumulates in the mitochondria, where it is a specific inhibitor of Complex I, binding at or near the ubiquinone binding site on ND1 and depleting ATP levels. The subsequent chain of events results in increased production of free radicals leading to cell death.[57]

A number of ND1 point mutations have been associated with the neurodegenerative disease Leber's Hereditary Optic Neuropathy.[58] Several of these have also been investigated in relation to PD, with ambiguous results.[59] However, one that was recently found to be associated with PD is a T→C missense mutation at nucleotide 4216 in ND1 (ND1/4216) (S. Kirchner, unpublished data). Given this finding and the effect of MPTP on ND1, investigation of these polymorphisms, particularly in relation to environmental exposures, seems warranted.

Studies which have examined relations between agricultural exposures and Parkinson's disease have produced inconsistent results. Some have observed an increased risk from farming[12,16] whereas others have not.[15,22] We are aware of only one study which has examined non-specific parkinsonism, as opposed to Parkinson's disease, in this context; it found increased risks of extrapyramidal signs among persons with a history of field or landscape work.[13] An association between well water use and Parkinson's disease has also been observed in some studies[9,19,27,30,49] but not in others.[12,15,18,23,24,28,29] Although many of these studies have examined associations between pesticides and Parkinson's disease, few specific chemicals or chemical classes have been identified as likely risk factors. Most of these studies have observed increased risk associated with pesticides,[6,7,10-12,15-18,23,25] but others have been negative.[20,21,27,28,30,49] Studies of paraquat and PD have produced similar results, with some reporting a positive association[14,18] and others reporting none.[15,21]

The present study examines MAO-B intron 13 and ND1/4216 polymorphisms, as well as interactions between pesticide exposure and those polymorphisms, in relation to risk of

parkinsonism. Subjects consist of a cohort of men occupationally exposed to pesticides, mostly as orchardists, in central Washington State.

METHODS

SUBJECTS

All subjects were participants in an earlier cohort study of men occupationally exposed to pesticides which was conducted between 1972 and 1976 by the Washington State Department of Health. Pesticide-exposed subjects in that study consisted primarily of orchardists (n=739, 56.8%), professional pesticide applicators (n=30, 2.3%), pesticide formulation plant workers (n=7, 0.5%), and other farm or agricultural workers (n=6, 0.5%). Non-exposed subjects were frequency-matched to exposed subjects by age, race, and work regimen (i.e., degree of physical activity). Subjects were male, mostly non-Hispanic Caucasian (95.0%), and aged 18 to 88 at the time they entered the original study.

Using information from various sources, including contact information provided by subjects in the original study, we were able to locate 482 surviving members of the original 1,300 subject cohort. They were invited in the summer of 1997 to participate in the current study and 323 (67.0%) accepted. They then underwent a 3-4 hour assessment at a centrally located testing center. The study protocol was approved by the University of Washington and Oregon Health Sciences University Human Subjects Committees, and all participants provided written, informed consent.

EXAMINATION

A neurologist-trained nurse administered an approximately 20 minute structured, uniform neurological examination to all subjects. The presence and severity of motor signs were recorded by the nurse using the United Parkinson's Disease Rating Scale (UPDRS).[40]

Parkinsonism was defined using the four cardinal signs: resting tremor, rigidity, bradykinesia, and postural reflex impairment. A sign was considered present if observed at a level of slight or greater on the UPDRS. Subjects were considered to have parkinsonism if they had two or more of these signs, or if they had one sign and were on anti-parkinsonian medication.

PESTICIDE USE ASCERTAINMENT

Each subject was asked to complete a self-administered questionnaire which asked detailed questions about the subject's use of pesticides (insecticides, herbicides, and fungicides) throughout his working career. The subject was asked to provide information on years of farming or occupational pesticide-related employment and, for discrete time periods: crops grown; number of acres of each crop; use (i.e., mixing, loading, or applying) of specific insecticides, herbicides, and fungicides (provided in a comprehensive list appropriate to the region); and target crops of each pesticide used. Most of the above information was solicited by calendar decade; use of specific pesticides was solicited by 5-year period as far back as 1960, and open-ended before then. The subject was also asked to report any pesticides not included in the list provided. Demographic information, including age, race, health conditions, use of medications, and history of alcohol consumption and smoking was solicited. An interviewer administered the questionnaire to the few subjects who were unable to read it for any reason.

GENETIC ASSAYS

Genomic DNA was extracted from a buccal cell swab collected from each subject at the time of examination. A PCR product was amplified from genomic DNA corresponding to a region of 742 bp that includes all of MAO-B intron 13, by using a Forward primer in exon 13 and a Reverse primer in exon 14. PCR primer sequences were as described by Kurth et al.[60] – Forward primer: 5'-GGATTTACTTTGCAGGCACC-3', Reverse primer: 5'-CAGACTCTGGTTCTGACTGC-3'. The amplified DNA underwent an allele-specific

ligation reaction followed by an Elisa using the protocol previously described by Tobe et al.[61] The sequences of the oligonucleotides used for ligation reaction were: 5'-TTAGAA-GAAAGATGGTGTCG-3' for allele G, 5'-TTAGAAGAAAGATGGTGTCA-3' for allele A, together with the common oligonucleotide 5'-CTTTTGCTATTTGCCAGTGTG-3'. Because MAO-B is X-linked and all subjects were male, they were classified as hemizygous G or A.

The OLA (oligonucleotide) assay for the ND1 T4216C mutation utilized 2 primers that were complementary to the consensus sequence surrounding the 4216 position. If no mutation was present, the annealed primers ligated and bound to strepavidin-coated plates via a biotin conjugate at one end of one primer, and were detected via a digoxygenin label at one end of the second primer. Because of sensitivity limits of the assay, subjects were classified as heteroplasmic/homoplasmic mutant (i.e., possessing a C nucleotide at position 4216) only if a large percentage of their mitochondrial DNA contained the mutation. Results were documented and stored using the Excel-based MacPearl program designed specifically for OLA analyses.

DATA ANALYSIS

Pesticide exposure was assessed in several ways. These included dichotomous measures of any history of 1) farm employment; 2) use of insecticides, herbicides, and fungicides, as well as carbamates, organophosphates, organochlorines, dithiocarbamates, and manganese-containing pesticides (which are also included in the dithiocarbamate category); and 3) use of specific pesticides such as azinphos methyl, DDT, ferbam, lead arsenate, mancozeb, maneb, methyl parathion, paraquat, TEPP, thiram, zineb, and ziram (most of which are contained in the above chemical classes). We also considered duration of exposure (in years) to the above factors. In addition, we examined history of well water consumption. Analyses involving continuous measures of exposure were restricted to subjects reporting some use of the appropriate chemical or chemical class in order to reduce heterogeneity in potential confounding factors. Specific pesticides and pesticide

classes were chosen for analyses based on their potential neurotoxicity (e.g., manganese-containing pesticides, dithiocarbamates which can produce carbon disulfide, paraquat because of its structural similarity to MPTP).

Prevalence ratio analyses were performed for MAO-B and ND1/4216 genotypes in relation to parkinsonism status using a generalized linear model with binomial distribution and a log link function.[41] Analyses stratified by genotype were also performed for certain demographic and pesticide exposure measures in relation to parkinsonism. Interactions were assessed on a multiplicative scale in models including both genotype and exposure metrics. Adjustment was made for age and pack-years of cigarette smoking. In all analyses, a 5% two-sided level of significance was used.

RESULTS

We observed no increased risk of parkinsonism associated with the MAO-B intron 13 A allele relative to the G allele (Table 12). We also found no association between ND1/4216 C or T polymorphisms and risk of parkinsonism. The prevalence of parkinsonism was roughly 20% for all genotypes.

There was no significant association between any smoking measure and parkinsonism for either the MAO-B A or G genotype, nor was there a significant interaction between these factors (Table 13). This was also true for ND1/4216 genotypes. Current smokers appeared to have a slightly higher risk of parkinsonism compared to non-smokers, but this was not significant and did not differ appreciably by genotype.

No significant associations were found between parkinsonism and a history of well water use, farm employment, or pesticide exposure within any genotype (Table 14). However, the number of subjects in many exposure/genotype subgroups was small, resulting in imprecise estimates and, in some cases, non-convergence of the models.

We observed no significant associations when parkinsonism was examined in relation to duration of the above measures within genotypes (Table 15). There was, however, a suggestion, statistically non-significant, of interaction between exposure to pesticides and ND1/4216 genotype, with a prevalence ratio of 3.0 (0.5, 19.2) for carriers of the C allele versus 0.9 (0.5, 1.6) for carriers of the T allele. This apparent interaction became more pronounced for exposure specifically to organophosphates, with prevalence ratios of 3.4 (0.8, 13.9) and 0.7 (0.4, 1.2) for carriers of the C and T alleles, respectively.

DISCUSSION

We found no association between either MAO-B intron 13 or ND1/4216 polymorphisms and parkinsonism in this cohort. We also observed no increased risk of parkinsonism from smoking or from any pesticide exposure, regardless of genotype (i.e., no gene-environment interaction). However, we did observe a suggestion of interaction between ND1/4216 genotype and exposure to pesticides on risk of parkinsonism, with a more pronounced effect observed for exposure to organophosphates.

Although we are unaware of any other studies which have examined genetic polymorphisms in relation to the general syndrome of parkinsonism, several have examined them in relation to Parkinson's disease (PD). Kurth et al.[60] observed a two-fold increase in PD risk associated with the MAO-B intron 13 A allele among Caucasians. A study by Ho et al.[62] found this allele to occur slightly more frequently in PD cases than controls in Caucasians, although the difference was not significant. A Japanese study[63] also found no significant association with this allele. In contrast, a study of Caucasians by Costa et al.[64] found an increased risk associated with the G allele. A recent case-control study of PD found an elevated odds ratio of 1.9 for male carriers of the ND1/4216 T→C missense mutation, although there was no increased risk for females (S. Kirchner, unpublished data). The lack of associations with genetic markers in the present study may be due to our focus on general parkinsonism as opposed to Parkinson's disease or to

differences in exposure or demographic characteristics between this and previous studies. Possible misclassification of parkinsonism status may also have masked any associations.

The discovery that 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced a PD-like syndrome[3] renewed interest in the search for environmental risk factors for PD. However, despite a great deal of research, very few environmental or genetic factors have been linked to PD. This has led some investigators to examine the possibility of differential genetic susceptibility to environmental toxicants in the pathogenesis of Parkinson's disease (gene-environment interaction).[5] One such study examined MAO-B in relation to cigarette smoking and found a reduced risk of PD related to pack-years of smoking among persons with the G allele, but the opposite relationship among persons with the A allele.[65] The present study did not observe this pattern. We found no inverse association between cigarette smoking and risk of parkinsonism associated with any allele. Most studies of smoking and PD without regard to genotype have found a protective effect from smoking.[42] Smokers in our study had comparable pack-years of smoking as smokers in studies reporting a protective effect.[43-45] However, to the best of our knowledge, smoking has not been examined in relation to other forms of parkinsonism. Smoking may have less pronounced effects on the slight parkinsonian signs exhibited by most of our subjects with parkinsonism. Alternatively, smoking may be less or non-protective against the factors contributing to parkinsonism in this population.

MAO-B bioactivates MPTP,[55] which is similar in chemical structure to the herbicide paraquat.[4] Some epidemiologic studies have reported an elevated risk of PD associated with paraquat,[14,18] whereas others have reported no effect.[15,21] A recent case-control study of gene-environment interaction involving Parkinson's disease, pesticides, and glutathione transferase (GST) polymorphisms[66] found a significant difference in distribution of GSTP1 genotypes between cases and controls exposed to pesticides. The present study found no association between parkinsonism and paraquat or other pesticides for any of the genotypes examined. Such discrepant findings may be due to

differences in study design, subject demographics, genetic susceptibilities, or local farming practices. Exposure misclassification may be partially responsible, with the possibility of recall bias producing spurious associations and random recall error masking true associations. Better retrospective exposure assessment or the use of prospective study designs may help resolve these issues.

One limitation of this study is possible misclassification of mitochondrial DNA genotype. Mitochondrial DNA can be heteroplasmic, in which there is a mixed intracellular population of wild type and mutant molecules. Because current sequencers identify only homoplasmic mutations, subjects identified as having the ND1/4216 mutation are those whose mitochondrial DNA sample contained a large proportion of the mutation. Some subjects heteroplasmic for the mutation may not have been identified. This would tend to dilute any observed effect.

In conclusion, we observed no association between parkinsonism and polymorphisms in either MAO-B intron 13 or ND1/4216. We also observed no interaction between these polymorphisms and smoking or pesticide exposure in relation to parkinsonism. Gene-environment interactions might, however, be detected for genetic polymorphisms other than those studied here.

Table 11. Selected demographics of subjects

Characteristic	n (%)				
	Total	Years of occupational pesticide exposure			
		0	1-30	31-50	> 50
	N=323	N=41	N=87	N=102	N=80
Age					
49-65	88 (27.2)	19 (46.3)	24 (27.6)	30 (29.4)	10 (12.5)
65-74	146 (45.2)	14 (34.1)	43 (49.4)	47 (46.1)	36 (45.0)
75-84	81 (25.1)	8 (19.5)	20 (23.0)	24 (23.5)	29 (36.3)
85-96	6 (1.9)	0 (0.0)	0 (0.0)	1 (1.0)	5 (6.3)
Race					
Caucasian, non-Hispanic	307 (95.0)	40 (97.6)	83 (95.4)	99 (97.1)	74 (92.5)
African American	1 (0.3)	0 (0.0)	0 (0.0)	1 (1.0)	0 (0.0)
Native American	6 (1.9)	1 (2.4)	2 (2.3)	0 (0.0)	3 (3.8)
Asian/Pacific Islander	4 (1.2)	0 (0.0)	1 (1.1)	2 (2.0)	1 (1.3)
Alcohol consumption					
Never or less than 1 drink/ month	138 (42.7)	14 (34.1)	39 (44.8)	44 (43.1)	35 (43.8)
Stopped > 6 months ago	34 (10.5)	7 (17.1)	11 (12.6)	5 (4.9)	8 (10.0)
Current drinker	148 (45.8)	20 (48.8)	37 (42.5)	52 (51.0)	37 (46.3)
Current cigarette smoker	29 (9.0)	5 (12.2)	7 (8.0)	8 (7.8)	8 (10.0)
Pack-years of cigarette smoking					
0	108 (33.4)	9 (22.0)	29 (33.3)	39 (38.2)	28 (35.0)
0.1 – 9.9	52 (16.1)	4 (9.8)	13 (14.9)	21 (20.6)	14 (17.5)
10.0 – 34.9	76 (23.5)	15 (36.6)	22 (25.3)	17 (16.7)	18 (22.5)
≥ 35.0	77 (23.8)	11 (26.8)	22 (25.3)	22 (21.6)	19 (23.8)
Physician-diagnosed Parkinson's disease (self-reported)	1 (0.3)	0 (0.0)	1 (1.1)	0 (0.0)	0 (0.0)

Table 12. Parkinsonism prevalences and prevalence ratios by genotype

	MAO-B		ND1/4216	
	G allele N=142	A allele N=167	T allele N=255	C allele N=66
	n (%) genotype		n (%) genotype	
Parkinsonism	29 (20.4)	35 (21.0)	55 (21.6)	12 (18.2)
No parkinsonism	112 (78.9)	132 (79.0)	200 (78.4)	53 (80.3)
	PR (95% CI) for parkinsonism		PR (95% CI) for parkinsonism	
Unadjusted	1.0	1.0 (0.7, 1.6)	1.0	0.9 (0.5, 1.5)
Adjusted*	1.0	1.0 (0.6, 1.5)	1.0	0.9 (0.5, 1.6)

* Adjusted for age and pack-years of smoking

Table 13. Association of parkinsonism with age and cigarette smoking by monoamine oxidase B (MAO-B) intron 13 and ND1/4216 genotypes

	Adjusted* PR (95% CI) for parkinsonism			
	MAO-B		ND1/4216	
Factor	A allele	G allele	C allele	T allele
Never smoker	1.0	1.0	1.0	1.0
Ever smoker†	0.6 (0.4, 1.2)	1.1 (0.6, 2.3)	1.0 (0.4, 2.8)	0.9 (0.5, 1.4)
Current smoker	1.8 (0.9, 3.8)	1.5 (0.6, 4.3)§	1.7 (0.5, 6.1)§	1.6 (0.9, 3.2)
Pack-years				
0.0	1.0	1.0	1.0	1.0
0.1-34.9	0.6 (0.3, 1.2)	1.3 (0.6, 2.7)	0.9 (0.3, 3.0)§	0.9 (0.5, 1.5)
≥ 35	0.8 (0.4, 1.7)	1.1 (0.5, 2.6)	1.1 (0.3, 3.9)§	0.9 (0.5, 1.7)

* Age analyses adjusted for pack-years of smoking; smoking analyses adjusted for age
† Defined as ever smoking 100 or more cigarettes
§ Less than 5 cases in at least one exposure group

Table 14. Association of parkinsonism with ever vs. never pesticide-related exposure by MAO-B intron 13 and ND1/4216 genotypes

	Adjusted* PR (95% CI) for parkinsonism – ever vs. never exposure			
	MAO-B		ND1/4216	
Exposure	**A allele**	**G allele**	**C allele**	**T allele**
Well water use	0.8 (0.4, 1.5)	0.8 (0.4, 1.7)	0.7 (0.2, 3.1)§	1.0 (0.6, 1.7)
Farm employment	1.5 (0.5, 4.2)§	1.2 (0.5, 3.2)§	-§	1.1 (0.5, 2.5)
Any pesticides	∞	0.3 (0.1, 1.5)§	0.5 (0.1, 3.2)§	-§
Any insecticides	0.9 (0.5, 2.0)	1.2 (0.6, 2.6)	-§	1.0 (0.6, 1.7)
Any herbicides	0.9 (0.5, 1.7)	0.9 (0.5, 1.6)	1.4 (0.4, 4.6)§	0.8 (0.5, 1.2)
Any fungicides	0.8 (0.5, 1.5)	0.9 (0.5, 1.7)	1.1 (0.3, 3.7)	0.8 (0.5, 1.3)
Any carbamates	0.7 (0.4, 1.2)	1.2 (0.6, 2.1)	1.4 (0.5, 4.3)	0.8 (0.5, 1.2)
Any organophosphates	0.9 (0.5, 1.9)	1.1 (0.5, 2.1)	0.9 (0.3, 2.7)§	0.9 (0.5, 1.5)
Any organochlorines	0.8 (0.4, 1.5)	1.0 (0.5, 1.9)	1.0 (0.3, 3.1)§	0.8 (0.5, 1.3)
Any dithiocarbamates	0.6 (0.3, 1.2)	1.1 (0.6, 2.1)	1.5 (0.5, 4.6)§	0.7 (0.4, 1.2)
Any manganese-containing	0.2 (0.0, 1.1)§	1.1 (0.4, 2.9)§	0.4 (0.1, 3.3)§	0.4 (0.1, 1.3)§
Azinphos methyl	0.7 (0.4, 1.5)	1.0 (0.5, 1.8)	1.2 (0.4, 3.8)§	0.7 (0.4, 1.2)
DDT	0.7 (0.4, 1.3)	1.0 (0.6, 2.0)	1.0 (0.3, 2.8)§	0.7 (0.5, 1.2)
Ferbam	0.0	2.8 (0.8, 9.7)§	3.1 (0.4, 27.1)§	0.5 (0.1, 3.1)§
Lead arsenate	1.2 (0.6, 2.4)	1.0 (0.5, 1.9)	-§	0.9 (0.6, 1.6)
Mancozeb	0.2 (0.0, 1.4)§	-§	0.0	0.5 (0.2, 1.5)§
Maneb	0.0	2.0 (0.3, 12.3)§	2.0 (0.2, 18.7)§	0.0
Methyl parathion	0.9 (0.5, 1.7)	0.7 (0.3, 1.3)	1.3 (0.4, 3.8)	0.6 (0.4, 1.0)
Paraquat	0.7 (0.3, 1.4)	1.1 (0.5, 2.1)	1.3 (0.4, 4.4)§	0.7 (0.4, 1.2)
TEPP	0.9 (0.4, 1.9)	1.0 (0.4, 2.1)	1.1 (0.3, 4.7)§	0.7 (0.4, 1.4)
Thiram	1.0 (0.3, 3.6)§	0.0	0.0	0.8 (0.2, 2.6)§
Zineb	0.0	-§	3.1 (0.4, 26.4)§	0.0
Ziram	0.7 (0.4, 1.4)	1.1 (0.6, 2.1)	1.6 (0.5, 4.8)§	0.8 (0.5, 1.4)

* Adjusted for age and pack-years of smoking

§ Less than 5 cases in at least one exposure group

Table 15. Association of parkinsonism with long vs. short duration of pesticide-related exposure[£] by MAO-B intron 13 and ND1/4216 genotypes

	Adjusted* PR (95% CI) for parkinsonism – long vs. short exposure duration[£]			
	MAO-B		ND1/4216	
Exposure	**A allele**	**G allele**	**C allele**	**T allele**
Well water use	2.7 (0.9, 8.0)[§]	0.7 (0.3, 1.5)	-[§]	1.1 (0.6, 2.1)
Farm employment	0.9 (0.5, 1.7)	-	-[§]	0.7 (0.5, 1.1)
Any pesticides	1.6 (0.8, 3.4)	0.9 (0.4, 1.8)	3.0 (0.5, 19.2)[§]	0.9 (0.5, 1.6)
Any insecticides	1.5 (0.7, 3.1)	0.9 (0.5, 1.8)	-[§]	1.0 (0.6, 1.8)
Any herbicides	1.8 (0.8, 3.9)	1.6 (0.7, 3.4)	1.1 (0.3, 4.2)[§]	1.6 (0.8, 3.1)
Any fungicides	1.4 (0.6, 3.3)	0.7 (0.3, 1.5)	1.9 (0.2, 20.0)[§]	1.1 (0.5, 2.2)
Any carbamates	0.7 (0.3, 1.8)	0.6 (0.2, 1.6)	-[§]	0.7 (0.4, 1.5)
Any organophosphates	1.1 (0.5, 2.1)	0.8 (0.4, 1.7)	3.4 (0.8, 13.9)[§]	0.7 (0.4, 1.2)
Any organochlorines	0.6 (0.3, 1.3)	-	0.5 (0.1, 2.5)[§]	0.8 (0.4, 1.3)
Any dithiocarbamates	0.8 (0.2, 3.2)[§]	0.5 (0.1, 2.1)[§]	1.4 (0.1, 14.9)[§]	0.6 (0.2, 1.5)
Any manganese-containing	∞[§]	0.0[§]	0.0[§]	-[§]
Azinphos methyl	0.7 (0.3, 2.0)	-[§]	-[§]	0.8 (0.3, 1.8)
DDT	1.4 (0.7, 3.2)	1.2 (0.6, 2.7)	0.8 (0.1, 7.0)[§]	1.6 (0.8, 3.1)
Ferbam	-[§]	-[§]	-[§]	-[§]
Lead arsenate	0.8 (0.3, 2.2)	-[§]	1.4 (0.3, 6.2)[§]	1.2 (0.6, 2.5)
Mancozeb	∞[§]	0.0[§]	-[§]	-[§]
Maneb	-[§]	0.0[§]	-[§]	-[§]
Methyl parathion	1.2 (0.5, 2.9)	-[§]	-[§]	1.1 (0.5, 2.4)
Paraquat	-[§]	0.8 (0.3, 2.4)[§]	1.1 (0.1, 17.6)[§]	0.7 (0.3, 1.7)
TEPP	1.0 (0.2, 4.3)[§]	2.4 (0.3, 22.0)[§]	0.0	1.6 (0.6, 4.9)[§]
Thiram	-[§]	-[§]	-[§]	-[§]
Zineb	-[§]	-[§]	-[§]	-[§]
Ziram	0.9 (0.2, 3.6)[§]	0.5 (0.1, 2.5)[§]	-[§]	0.6 (0.2, 1.5)

* Adjusted for age and pack-years of smoking

§ Less than 5 cases in at least one exposure group

£ Restricted to subjects with the particular exposure;
Range of years for short (≤ median) and long (> median) duration categories, respectively – Well water use: 1-45, 46-89; Farm employment: 1-41, 42-80; Pesticides: 1-41, 42-81; Insecticides: 1-38, 39-76; Herbicides: 1-22, 23-55; Fungicides: 1-27, 28-73; Carbamates: 1-20, 21-41; Organophosphates: 1-30, 31-51; Organochlorines: 1-26, 27-55; Dithiocarbamates: 1-17, 18-57; Manganese-containing: 1-12, 31-37; Azinphos methyl: 1-20, 21-40; DDT: 1-15, 16-51; Ferbam: 1-5, 6-50; Lead arsenate: 1-24, 25-44; Mancozeb: 1-12, 13-37; Maneb: 1-7, 8-30; Methyl parathion: 1-15, 16-45; Paraquat: 1-15, 16-39; TEPP: 1-10, 11-51; Thiram: 1-10, 11-33; Zineb: 1-5, 6-15; Ziram: 1-17, 18-57

CHAPTER 4: VALIDITY STUDY OF SELF-REPORTED PESTICIDE EXPOSURE AMONG ORCHARDISTS

ABSTRACT

Objective: To examine the accuracy of recall of historical pesticide use among orchardists. *Methods:* All 185 orchardists in this study had previously participated in a cohort study of men occupationally exposed to pesticides. In that study, which took place from 1972 to 1976, subjects were interviewed annually and asked to list the pesticides which they had used since the previous interview. In 1997, subjects were re-contacted and asked to complete a self-administered questionnaire which asked detailed questions about their lifetime use of pesticides. Considering the 1972-76 data as the standard, sensitivity and specificity of recall were calculated for certain pesticides and pesticide categories. *Results:* Subjects had a mean age of 70.8 years (range: 49-96; standard deviation: 8.4). They had farmed an average of 47.8 years (range: 8-80). Insecticide, herbicide, and fungicide use was reported by 100.0%, 89.7%, and 85.9% of subjects, respectively. Average lifetime number of pesticides used was 18. Sensitivity of pesticide recall was good to excellent (0.6-0.9) for broad categories such as insecticides, herbicides, and fungicides, for heavily used chemical classes such as organophosphates and organochlorines, and for commonly used pesticides; it was lower and more variable (0.1-0.6) for other pesticide categories. Recall specificity was highest (0.7-0.9) for the least used pesticides and chemical classes, such as dithiocarbamates and manganese-containing pesticides, and was generally modest for the rest (0.5-0.6). *Conclusion:* Recall accuracy was good for commonly used pesticides and pesticide categories. This level of recall accuracy is probably adequate for epidemiologic analyses of broad categories of pesticides, but is a limitation for detecting more specific associations.

INTRODUCTION

Self-report is often the only means of assessing historical exposure in epidemiologic studies. However, concerns about the validity of such self-reported exposures arise when the exposures vary qualitatively and quantitatively over time, are not particularly memorable, or when the time lag between exposure and reporting is great.[67,68] Reporting error can result in either over- or under-estimation of the health effects of a given exposure, depending on whether the reporting error is differential or non-differential.

We are aware of only one study that has investigated the accuracy of self-reported historical pesticide use among farmers. Blair and Zahm,[69] in a study of mostly grain farmers, found generally good agreement between farmers and suppliers for reported use of pesticides. They also found that these farmers reported lifetime use of very few pesticides.

Several other studies have examined the quality of self-reported work histories[70-76] and occupational exposure histories[74,77-79] provided by non-farmers. These studies have found that self-reported work histories are reasonably accurate when compared to company or government records. However, while recall of occupational exposures is generally fair to good, it is highly variable. One recurring finding throughout these studies is that recall decreases as the number of jobs or assignments held by subjects increases.[70,72-76] In addition, validity or reliability of recall is higher for the fact of employment than for the dates of that employment.[71,74,75]

The present study examined recall of pesticide use among a cohort of farmers, mostly orchardists, in central Washington state. All subjects were participants in an earlier cohort study of men occupationally exposed to pesticides which was begun in the early 1970s by the Washington State Department of Health. Subjects in the present study completed a detailed questionnaire eliciting data on demographics, lifestyle factors, and lifetime farming and pesticide practices. This information was used to examine agreement between the pesticides reported originally by these orchardists in the early to mid 1970s

with those reported in 1997 for the same time period. This study is part of a larger investigation of possible neurological effects from long-term pesticide exposure.

METHODS

SUBJECTS

The location and recruitment of subjects have been described elsewhere (see Chapter 1). In brief, all subjects had previously participated in a cohort study carried out by the Washington State Department of Health from 1972 through 1976. Pesticide-exposed subjects in that study consisted primarily of orchardists (n=739, 56.8%), professional pesticide applicators (n=30, 2.3%), pesticide formulation plant workers (n=7, 0.5%), and other farm or agricultural workers (n=6, 0.5%). Non-exposed subjects were frequency-matched to exposed subjects by age, race, and work regimen (i.e., degree of physical activity). All subjects were males, mostly non-Hispanic Caucasian (95.0%), and aged 18 to 88 at the time they entered that study.

Using information from various sources, including contact information provided by subjects in the original study, we were able to locate 482 surviving members of the original 1,300 subject cohort. They were invited in the summer of 1997 to participate in a follow-up study, and 323 (67.0%) accepted. Study participants underwent a 3-4 hour assessment at a centrally located testing center. The study protocol was approved by the University of Washington and Oregon Health Sciences University Human Subjects Committees, and all participants provided written, informed consent.

In order to minimize heterogeneity of the study population, and potential bias, subjects in the present study were restricted to the 185 persons reporting their primary occupation as orchardist in any of the original interviews. All other subjects were excluded.

PESTICIDE USE ASCERTAINMENT

In the original study, conducted from 1972 through 1976, subjects were interviewed approximately once each year. As part of each interview, each subject was asked to list up to five agricultural chemicals to which he had had substantial exposure since the previous interview.

In the follow-up study in 1997, each subject was given a self-administered questionnaire. This questionnaire asked detailed questions about the subject's use of pesticides throughout his farming/work career. The subject was asked to provide information on years of farming or occupational pesticide-related employment and, for discrete time periods: crops grown; number of acres of each crop; and use (i.e., mixing, loading, or applying) of specific insecticides, herbicides, and fungicides (provided in a comprehensive list appropriate to the region). Pesticide use information was solicited by 5-year period as far back as 1960, and open-ended before then. The subject was also asked to provide information on any pesticides not included in the list provided.

DATA ANALYSIS

Since the pesticide use information reported in the original interviews was collected at the time those pesticides were being used, it was assumed to be reasonably accurate and was treated as the "gold standard" in all analyses. Sensitivity was calculated as the proportion of those subjects who had reported using a particular agent in the original study who also reported using it in the follow-up study (during the appropriate time period). Specificity was calculated as the proportion of those subjects who had not reported using a particular agent in the original study who reported having not used it in the follow-up study.

Sensitivity and specificity were calculated for use of 1) pesticides generally, 2) the insecticide, herbicide, and fungicide functional classes; 3) the organophosphate, organochlorine, dithiocarbamate, and manganese-containing pesticide (subset of dithiocarbamate) chemical classes; and 4) specific pesticides such as azinphos methyl,

DDT, ferbam, lead arsenate, mancozeb, maneb, methyl parathion, paraquat, TEPP, thiram, zineb, and ziram (selected because of their potential neurotoxicity). The categories in items 1, 2, and 3 above were created by grouping the appropriate individually-reported pesticides. Analyses were limited to those chemicals whose use was reported by 10 or more subjects in the original study. Carbamates were not analyzed for this reason.

Because the original study solicited pesticide use information annually during the years 1972-76, whereas the follow-up study solicited this information in 5-year blocks over a lifetime, one set of analyses compared data from 1972-74 in the original study to data from 1970-74 in the follow-up study. In order to examine the accuracy of exposure recall while allowing for some error in the timing of exposure, another set of analyses compared 1972-76 in the original study to 1965-79 (the smallest incremental increase possible with our data) in the follow-up study. Lastly, to assess accuracy of recall of ever/never exposure, analyses were also conducted comparing 1972-76 in the original study to lifetime use in the follow-up study.

All analyses were restricted to subjects in the current study. Comparisons of stratified sensitivities and specificities were done using a chi-square test with 1 df. In all statistical tests, a 5% two-sided level of significance was used.

RESULTS

The 185 subjects in this study had a mean age of 70.8 years in 1997, ranging from 49 to 96 (Table 16). All subjects were male; almost all were non-Hispanic Caucasian (95.1%). They had farmed an average of 47.8 years (range: 8-80). Most (56.5%) were still farming at the time of the follow-up interview; 21.5% had stopped farming within the preceding 9 years.

In the original study, these subjects reported use of an average of 5.4 different pesticides during 1972-74, consisting of 4.9 insecticides, 0.2 herbicides, and 0.5 fungicides (Table

17). These numbers were somewhat higher for 1972-76, with a total reported use of 6.3 pesticides, which included 5.7 insecticides, 0.3 herbicides, and 0.6 fungicides. In the follow-up study, subjects appeared to over-report pesticide use during this approximate time period, reporting an average of 8.8 pesticides used from 1970-74, consisting of 5.5 insecticides, 1.5 herbicides, and 1.8 fungicides. The number of pesticides used in each 5-year period steadily increased between 1965 and 1979.

Temporal and point patterns of pesticide usage by these subjects as reported in both the original and follow-up studies are shown in Table 18. In the original study, insecticide use was reported by 97.3% of subjects; one or more insecticides was reported in 94.7% of interviews, and insecticides accounted for 91.1% of all pesticides reported. Herbicide use was much lower, reported by only 23.1% of subjects, mentioned in only 11.4% of interviews, and accounting for only 2.5% of pesticides reported. Fungicide use was also much lower, reported by 47.3% of subjects in 24.7% of interviews, and accounting for only 5.7% of all pesticides. Organophosphates and organochlorines were widely used, reported by 96.2% and 80.1% of subjects, respectively. However, although organophosphates accounted for 51.6% of pesticides reported, organochlorines accounted for only 18.5%. Dithiocarbamates and manganese-containing pesticides were used by less than one quarter of subjects and accounted for only about 3% of all pesticides reported. Individual pesticide use ranged from a high of 84.4% of subjects and 16.9% of pesticides reported for parathion to a low of 0% of subjects for DDT.

More subjects reported using insecticides during the original study than reported using them around that general time period in the follow-up study (Table 18). In contrast, fewer subjects in the original study reported using herbicides and fungicides than in the follow-up study. Reporting was also higher in the original study for use of organophosphates, organochlorines, and manganese-containing pesticides, but was somewhat lower for dithiocarbamates. Reporting of specific pesticides in the original study was also much higher than in the follow-up study for the most commonly reported pesticides in the original study. Reporting in the original study was frequently lower than in the follow-up

study for the least commonly reported pesticides in the original study. This is illustrated most dramatically for DDT, which was not reported by any subjects in the original study, but was reported by almost one third of subjects in the follow-up study as being used during that period.

All subjects reported in the follow-up study using one or more pesticides on their crops at some time (Table 19). All had used insecticides, 89.7% had used herbicides, and 85.9% had used fungicides. Ninety three percent reported using five or more insecticides, 35.7% five or more herbicides, and 43.2% five or more fungicides. The median lifetime number of pesticides used was 18.

In comparisons between the 1972-74 period in the original study and the 1970-74 period in the follow-up study, sensitivity was good to very good for use of any pesticides (0.94), insecticides (0.90), herbicides (0.66), and fungicides (0.62) (Table 20). However, specificity was fair to poor, ranging from 0.25 for insecticides to 0.49 for fungicides. Sensitivity was high for use of organophosphates (0.90), but lower for organochlorines (0.60) and dithiocarbamates (0.42), and very low for manganese-containing pesticides (0.09). Specificities ranged from 0.27 for organophosphates to 0.90 for manganese-containing pesticides. Sensitivities for particular pesticides varied greatly from a high of 0.72 for parathion to a low of 0.22 for phosmet; specificities ranged from 0.82 for phosmet to 0.48 for diazinon (Figure 1). In general, sensitivity was directly related and specificity inversely related to how widely and frequently a chemical or chemical class was used (as shown in Table 18).

When we widened the matching intervals to 1965-79 in the follow-up study and 1972-76 in the original study, all the sensitivities increased or remained the same while all the specificities decreased (Table 20). The greatest increase in sensitivity was observed for use of herbicides, organochlorines, azinphos methyl, diazinon, and phosmet. Specificity decreased most for organochlorines, DDT, diazinon, paraquat, and parathion. Most of these changes had a modest effect on interpretation of sensitivity and specificity estimates.

When we considered *ever* use of these chemicals reported in the follow-up study compared to 1972-76 in the original study, all sensitivities increased and specificities decreased appreciably (Table 20). Sensitivities became very high for pesticide functional classes (range: 0.87-1.00) and for organophosphates (0.99) and organochlorines (0.97). Sensitivity was good for dithiocarbamates (0.67) and fair for manganese-containing pesticides (0.41). It was good to excellent for most individual pesticides. This general pattern persisted in all subsequent stratified sensitivity and specificity analyses.

In general, sensitivity was similar to moderately higher among orchardists 70 years of age or younger compared to those over 70, although few differences were statistically significant (Table 21). Specificity was generally similar. However, while sensitivity in the younger group was good to very good for many general pesticide categories, it was lower and quite variable for individual pesticides.

Because in the original study, subjects could report no more than five pesticides per interview, low specificities may partially reflect limits in the original interviews rather than poor recall on the part of the orchardists (i.e., some "false positives" could represent correct retrospective reporting of pesticides that the subject was unable to report in the original interviews because of the five pesticide reporting limit in those interviews). In fact, 76% of orchardists reported five pesticides in at least one interview. Therefore, sensitivity analyses were performed comparing those orchardists reporting five pesticides in any interview to those reporting fewer than five in all interviews (Table 22). The number of subjects in these two categories, particularly in the group consistently reporting fewer than five pesticides per interview, was small for several chemicals; some estimates were consequently unstable. As expected, there was a general pattern of higher specificity among those orchardists consistently reporting fewer than five pesticides per interview. On the other hand, sensitivity tended to be lower in this group.

Sensitivity analyses stratified by lifetime number of pesticides used revealed that for orchardists reporting use of more than 20 pesticides, sensitivity was almost always appreciably higher and specificity similarly lower compared to those reporting 20 or less

(data not shown). However, there was a strong inverse association between age and lifetime number of pesticides used, and when number of pesticides was further stratified by age, this difference disappeared. Additional analyses comparing subjects with 50 or fewer years of farming to those with more produced estimates that were generally similar in both groups. And results from analyses restricted to subjects still engaged in farming were similar to those calculated for all subjects.

DISCUSSION

The purpose of this study was to assess the validity of self-reported retrospective pesticide use among orchardists. We found that recall of pesticide use 21-25 years prior was highly variable. Sensitivity was good for general pesticide categories such as insecticides, herbicides, and fungicides. It was more variable for chemical classes (e.g., organophosphates, organochlorines) and for specific pesticides. Recall tended to be better in younger subjects.

Orchardists in this study reported more pesticide use than farmers elsewhere. Blair and Zahm,[69] in a study of primarily grain farmers, found that 83% of farmers reported ever using insecticides, 54% herbicides, and 9% fungicides during their lifetimes. Few subjects reported use of five or more from any one of those groups. In contrast, the vast majority of orchardists in the present study reported using chemicals from all of those groups. The median number of pesticides used by orchardists in the present study was 18, and one quarter reported using 28 or more. This discrepancy between studies is most likely due to differences in study populations, since subjects in the present study were predominantly apple orchardists and apples are one of the most pesticide-intensive crops in the United States.[80]

The complexity and variability described here is likely to decrease accuracy of pesticide use recall, especially after long periods of time. This recall error can either exaggerate or attenuate estimated pesticide-disease associations when it is differential and will attenuate such associations when it is random, or non-differential.[81] Analyses stratified by

parkinsonism status (as defined in Chapter 1) and age found that sensitivity and specificity tended to be similar for cases and non-cases. Thus, in the parent study of parkinsonism and pesticide exposure, such erroneous recall is likely to be non-differential.

The fact that over-reporting was slight for insecticides but pronounced for herbicides and fungicides suggests that this bias may be of concern primarily in certain situations. At the time of the original study, insecticide use in this population was already well established, but herbicides and fungicides were only beginning to become prominent. The data indicate that this general time period saw particularly rapid growth in the use of these chemicals. Widening the comparison time period in the follow-up study produced more marked proportional increases in the sensitivities for herbicides and fungicides than for insecticides. Thus, over-reporting may be of greatest concern when examining a narrow time interval in a period of rapid change. Unfortunately, this study lacked the necessary data to test this hypothesis.

The low to moderate recall sensitivities for specific pesticides suggest the limitations of long-term recall of pesticide use among farmers using large numbers of pesticides. In general, the highest sensitivities were observed for those pesticides which were most widely and frequently used. A farmer is probably less likely to err in reporting the fact of use of a pesticide than in the temporal pattern of that use. In fact, we observed a general increase in sensitivity as the window was widened around the time period of interest (i.e., from 1970-74 to 1965-79). Given the appreciable number of pesticides used by these subjects and the frequently-changing regulations, effectiveness, and economics of pesticides, it is not surprising that these orchardists would have difficulty recalling exactly when they started and stopped using particular ones.

The true recall specificities are probably higher than our estimates suggest. There are two reasons for this. The first is the approximation of time frames between the two studies. The most appropriate comparison was between 1972-74 of the original study and 1970-74 of the follow-up study. However, pesticides which subjects reported using during

1970-74 in the follow-up study but which were not reported during 1972-74 in the original study (apparent "false positives") may simply be ones which were used during the first, but not latter, part of the 1970-74 time period. In fact, most subjects who reported such false positives also indicated use of these pesticides in 1965-69. However, in many cases, with the notable exception of DDT (which was banned in the United States in 1973), these subjects also reported use of the pesticides in 1975-79.

In addition, the original study solicited and recorded at each interview a maximum of five pesticides used by each subject. There was a substantial proportion of subjects (76%) who listed five pesticides in at least one interview and a high proportion who did this multiple times. It is possible that these subjects, in fact, used more than those five pesticides during the relevant time period, but reported only five because of design limitations of the original interviews. Therefore, there may be some pesticide use correctly reported by subjects in the follow-up study which was not reported/recorded in the original study for this reason. These erroneous "false positives" would also reduce the estimated recall specificity.

It is possible that some subjects forgot to report use of some pesticides in one or more of the original interviews. There is no way to determine the extent to which this might have occurred. If these non-reported pesticides were used intermittently or were consistently not reported in the original study, but were reported in the follow-up study, then estimated specificities could be reduced. We believe that this situation is more likely than the converse, in which a subject were to report in the original study the use of a pesticide that he had not used, but not report this in the follow-up study; this would tend to reduce sensitivity.

Confusion around pesticide names can lead to a decrease in both sensitivity and specificity. Most pesticides have several names, and some have many. We attempted to list several of the most common ones for each pesticide; however, a subject might have used a particular chemical under a brand name not listed or he might have missed a name he knew while scanning through a list of unfamiliar names. This would result in under-

reporting and a consequent reduction in sensitivity. It is also possible that some subjects confused similar names of different pesticides and mistakenly reported ones that they had not used. This would result in over-reporting, with a resultant reduction in specificity.

One limitation of this study was the narrow time frame in which pesticide use data were originally collected. Having only a five year period from 1972 to 1976 prevented us from more closely matching the comparison time frames, thus artificially reducing agreement between data from the two studies. In addition, it limits the generalizability of our results since recall accuracy tends to be related to time elapsed since the event being recalled. One would expect recall to be better for more recent time periods. However, a caveat to this is that the number of pesticides used in any given period has increased over time, thus creating greater complexity which might adversely affect recall.

The generalizability of our findings to other orchardists may be limited since participation of these subjects in the original study might have improved their recall in the follow-up study. However, the original study ended over twenty years prior to the follow-up study, and it is unlikely that any appreciable memory booster effect would have persisted.

Our results are comparable to those of Blair and Zahm,[69] whose study comparing farmers' recall of pesticide use to information provided by pesticide suppliers observed agreement of about 60% for use of insecticides and herbicides. We also observed similar patterns to those found in studies examining the quality of self-reported work or occupational exposure histories among non-farmers. Joffe[78] observed that sensitivity improved while specificity worsened among workers in the printing and plastics industries when exposures were described in more general terms. Other researchers have found that accuracy of recall was higher for the fact of employment than for the dates of that employment.[71,74,75] Our data suggest that farmers are better able to recall the fact of use of a given pesticide than its specific years of use.

In conclusion, we found pesticide use to be high in this cohort of elderly orchardists, with many reporting use of a large number of different pesticides over their lifetimes. There appeared to be a modest over-reporting bias during the comparison period, particularly for herbicides and fungicides. Sensitivity of pesticide recall was good to excellent for broad categories such as insecticides, herbicides, and fungicides, for certain heavily used chemical classes such as organophosphates and organochlorines, and for commonly used pesticides. It tended to be fair to poor for less frequently used pesticides and chemical classes. Sensitivity increased as the time frame was widened, becoming generally good to excellent for ever/never use. Recall specificity was highest for the least used pesticides and chemical classes, such as dithiocarbamates and manganese-containing pesticides, and was generally modest for the rest. This level of recall accuracy is probably adequate for epidemiologic analyses of broad categories of pesticides, but is a limitation for detecting more specific associations.

Table 16. Selected demographics of subjects

Characteristic	**n (%) N=185**
Age in 1997	
49-65	40 (21.5)
65-74	82 (44.3)
75-84	57 (30.8)
85-96	6 (3.2)
Race	
Caucasian, non-Hispanic	176 (95.1)
African American	1 (0.5)
Native American	4 (2.2)
Asian/Pacific Islander	3 (1.6)
Years of farming	
1-30	21 (11.4)
31-50	89 (48.1)
> 50	75 (40.5)
Years since retirement from farming	
0 (still farming)	105 (56.5)
1-9	40 (21.5)
≥ 10	40 (21.5)

Table 17. Number of pesticides reported in original and follow-up studies by time period

	Mean (SD)			
	Total	**Insecticide**	**Herbicide**	**Fungicide**
Original study				
1972-74	5.4 (1.8)	4.9 (1.7)	0.2 (0.5)	0.5 (0.7)
1975-76	4.2 (1.5)	4.0 (1.5)	0.1 (0.4)	0.3 (0.5)
1972-76	6.3 (1.9)	5.7 (1.8)	0.3 (0.6)	0.6 (0.7)
New study				
1965-69	6.4 (6.1)	4.3 (4.0)	0.9 (1.4)	1.2 (2.0)
1970-74	8.8 (7.4)†	5.5 (4.6)	1.5 (2.0)†	1.8 (2.5)†
1975-79	9.6 (8.0)	5.7 (4.7)	2.0 (2.3)	2.0 (2.6)
1965-79	12.1 (8.6)‡	7.4 (4.9)‡	2.3 (2.4)‡	2.4 (2.9)‡
Total	20.2 (10.9)	11.7 (5.6)	3.8 (2.7)	4.6 (3.9)

† $p < 0.05$ compared to 1972-74
‡ $p < 0.05$ compared to 1972-76

Table 18. Selected pesticide usage patterns reported in original and follow-up studies

Pesticide	Original study use reported – 1972-76			Follow-up study use reported – % of subjects		
	% of subjects	% of interviews	% of pesticides	1965-69	1970-74	1975-79
Any pesticide	97.8	95.1	100.0	86.0	92.5	91.9
Any insecticide	97.3	94.7	91.1	82.8	88.7	88.2
Any herbicide	23.1	11.4	2.5	41.4	55.9	65.6
Any fungicide	47.3	24.7	5.7	45.7	55.9	59.1
Any organophosphate	96.2	90.4	51.6	78.5	88.7	88.2
Any organochlorine	80.1	60.6	18.5	57.5	58.6	59.1
Any dithiocarbamate	23.7	12.6	3.2	19.4	31.2	33.3
Any manganese-containing	21.5	11.2	2.8	4.8	9.1	11.8
Azinphos methyl (Guthion)	71.5	54.1	13.5	24.7	34.9	43.5
Carbaryl (Sevin)	32.3	16.5	4.1	36.0	43.5	48.4
DDT	0.0	0.0	0.0	41.4	31.2	17.2
Diazinon	37.6	25.0	6.3	41.9	47.3	48.4
Endosulfan (Thiodan)	73.7	52.5	13.1	22.6	33.9	36.0
Ethylan (Perthane)	31.2	16.6	4.2	9.7	14.5	19.4
Oxythioquinox (Morestan)	39.2	23.6	5.9	15.6	24.2	22.0
Paraquat	7.0	2.8	0.7	16.1	21.5	32.8
Parathion	84.4	67.6	16.9	53.8	62.9	57.5
Phosmet (Imidan)	34.4	18.7	4.7	10.2	12.9	14.5
Phosphamidon	10.8	5.1	1.3	9.1	15.6	19.9

Table 19. Lifetime pesticide use reported in follow-up study

Type or number of pesticides	**Number (%) of subjects N=185**
Any pesticide	185 (100.0)
Any insecticide	185 (100.0)
Any herbicide	166 (89.7)
Any fungicide	159 (85.9)
Only one insecticide	2 (1.1)
Only one herbicide	12 (6.5)
Only one fungicide	17 (9.2)
≥ 5 insecticides	172 (93.0)
≥ 5 herbicides	66 (35.7)
≥ 5 fungicides	80 (43.2)
Number of pesticides reported	
0	0 (0.0)
1-10	36 (19.5)
11-20	67 (36.2)
21-30	46 (24.9)
31-40	28 (15.1)
> 40	8 (4.3)

Table 20. Sensitivity and specificity of pesticide use recall for different time periods in both the original and follow-up studies

Pesticide	Follow-up study time period vs. original study time period					
	1970-74 vs. 1972-74		1965-79 vs. 1972-76		Ever vs. 1972-76	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
Any pesticide	0.94	(0.29)*	0.97	(0.00)	1.00	(0.00)
Any insecticide	0.90	(0.25)	0.95	(0.00)	1.00	(0.00)
Any herbicide	0.66	0.46	0.89	0.32	0.97	0.16
Any fungicide	0.62	0.49	0.70	0.40	0.87	0.18
Any organophosphate	0.90	0.27	0.96	(0.00)	0.99	(0.00)
Any organochlorine	0.60	0.46	0.77	0.27	0.97	0.08
Any dithiocarbamate	0.42	0.71	0.48	0.65	0.67	0.46
Any manganese-containing	0.09	0.90	0.19	0.87	0.41	0.77
Azinphos methyl (Guthion)	0.49	0.55	0.68	0.43	0.86	0.24
Carbaryl (Sevin)	0.59	0.59	0.69	0.48	0.80	0.29
DDT	-	0.68	-	0.51	-	0.16
Diazinon	0.48	0.48	0.71	0.31	0.94	0.14
Endosulfan (Thiodan)	0.55	0.65	0.63	0.51	0.86	0.26
Ethylan (Perthane)	0.39	0.84	0.40	0.68	0.58	0.58
Oxythioquinox (Morestan)	0.43	0.68	0.43	0.61	0.58	0.47
Paraquat	(0.56)	0.73	0.75	0.50	0.92	0.29
Parathion	0.72	0.64	0.79	0.46	0.91	0.25
Phosmet (Imidan)	0.22	0.82	0.40	0.80	0.53	0.60
Phosphamidon	(0.44)	0.79	0.56	0.71	0.76	0.54

* Values are shown in parentheses if they are based on fewer than 10 subjects in that category.

Table 21. Sensitivity and specificity of pesticide use recall by age, comparing follow-up study 1970-74 to original study 1972-74

Pesticide	Sensitivity[£]		Specificity[£]	
	≤ 70	> 70	≤ 70	> 70
Any pesticide	0.93	0.95	(0.20)[*]	(0.50)
Any insecticide	0.89	0.91	(0.17)	(0.50)
Any herbicide	0.54	0.74	0.45	0.46
Any fungicide	0.68	0.54	0.47	0.50
Any organophosphate	0.89	0.92	(0.29)	(0.25)
Any organochlorine	0.62	0.59	0.48	0.43
Any dithiocarbamate	0.53	0.32	0.70	0.71
Any manganese-containing	0.13	0.06	0.90	0.91
Azinphos methyl (Guthion)	0.64	0.39[†]	0.54	0.56
Carbaryl (Sevin)	0.70	0.50	0.56	0.62
DDT	-	-	0.66	0.69
Diazinon	0.45	0.50	0.45	0.51
Endosulfan (Thiodan)	0.65	0.48	0.57	0.70
Ethylan (Perthane)	0.60	0.15[†]	0.89	0.80
Oxythioquinox (Morestan)	0.64	0.29[†]	0.70	0.67
Paraquat	(0.50)	(0.57)	0.77	0.70
Parathion	0.73	0.70	0.62	0.67
Phosmet (Imidan)	0.31	0.17	0.77	0.88
Phosphamidon	(0.00)	(0.57)	0.75	0.82

[£] Stratified by age: ≤ 70, > 70

[*] Values are shown in parentheses if they are based on fewer than 10 subjects in that category.

[†] $p < 0.05$ for chi-square test between strata

Table 22. Sensitivity and specificity of pesticide use recall by maximum number of pesticides reported in *any* interview, comparing follow-up study 1970-74 to original study 1972-74

Pesticide	Sensitivity[£]		Specificity[£]	
	< 5	= 5	< 5	= 5
Any pesticide	0.87	0.96	(0.17)[*]	(1.00)
Any insecticide	0.79	0.93[†]	(0.14)	(1.00)
Any herbicide	(0.75)	0.64	0.56	0.42
Any fungicide	0.70	0.61	0.71	0.38[†]
Any organophosphate	0.81	0.93[†]	(0.13)	(0.67)
Any organochlorine	0.61	0.60	0.52	0.37
Any dithiocarbamate	(1.00)	0.38	0.86	0.64[†]
Any manganese-containing	(0.00)	0.10	0.98	0.87
Azinphos methyl (Guthion)	0.33	0.52	0.60	0.50
Carbaryl (Sevin)	(0.14)	0.68[†]	0.54	0.61
DDT	-	-	0.77	0.64
Diazinon	0.43	0.49	0.50	0.48
Endosulfan (Thiodan)	0.46	0.57	0.75	0.54
Ethylan (Perthane)	(0.50)	0.38	0.79	0.86
Oxythioquinox (Morestan)	(0.17)	0.48	0.68	0.68
Paraquat	(1.00)	(0.50)	0.86	0.68[†]
Parathion	0.59	0.75	0.67	0.61
Phosmet (Imidan)	(0.00)	0.26	0.81	0.83
Phosphamidon	(0.33)	(0.50)	0.86	0.76

[£] Stratified by maximum number of pesticides reported in *any* interview: < 5, = 5

[*] Values are shown in parentheses if they are based on fewer than 10 subjects in that category.

[†] $p < 0.05$ for chi-square test between strata

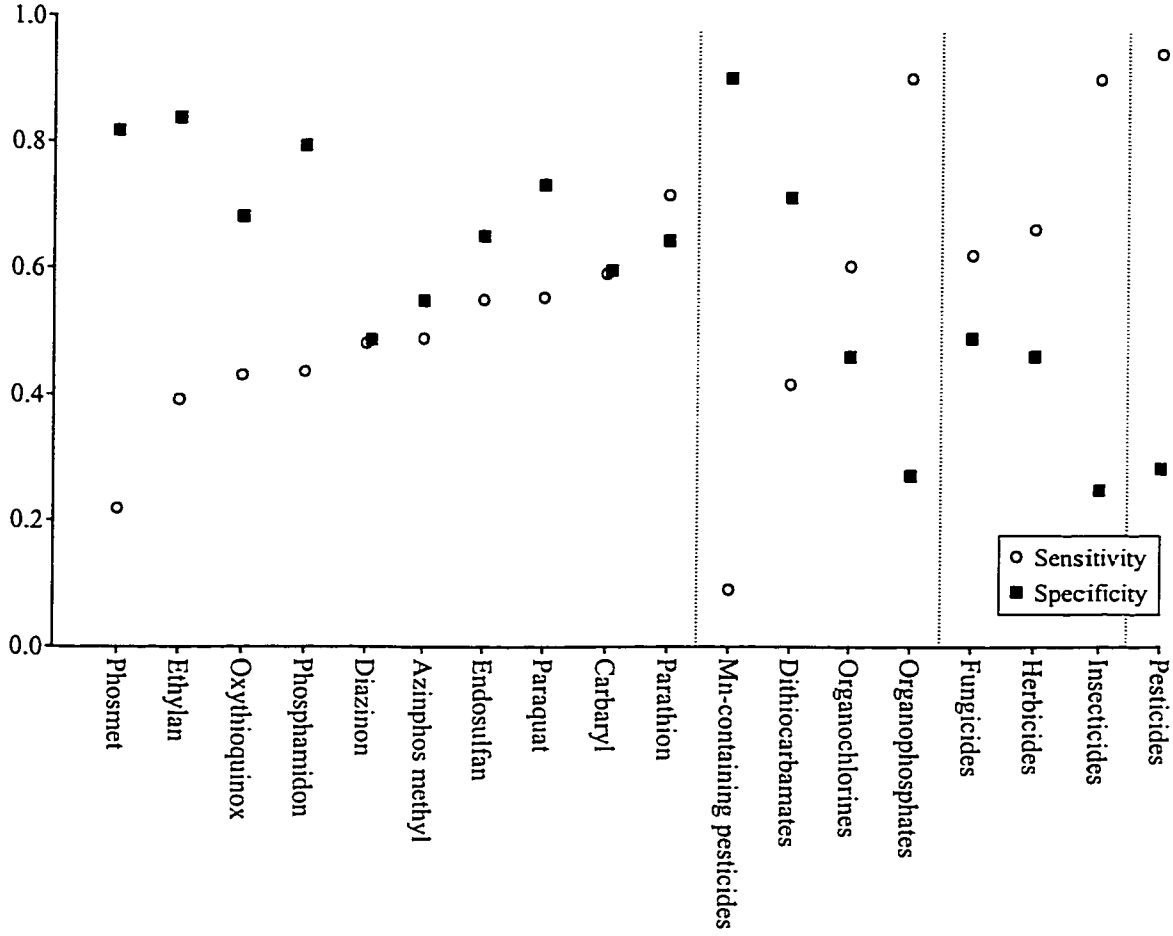

Figure 1. Sensitivities and specificities of pesticide use recall, comparing follow-up study 1970-74 to original study 1972-74, ordered by increasing sensitivity within groups of increasing generality.

BIBLIOGRAPHY

1. Tanner CM. Occupational and environmental causes of parkinsonism. Occup Med 1992;7(3):503-13.

2. Langston JW, Irwin I, Ricaurte GA. Neurotoxins, parkinsonism and Parkinson's disease. Pharmacol Ther 1987;32(1):19-49.

3. Langston JW, Ballard P, Tetrud JW, Irwin I. Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. Science 1983;219(4587):979-80.

4. Barbeau A, Roy M, Cloutier T, Plasse L, Paris S. Environmental and genetic factors in the etiology of Parkinson's disease. In: Yahr MD, Bergmann KJ, eds. Parkinson's disease. Advances in neurology. Vol. 45. New York: Raven Press, 1987;299-306.

5. Checkoway H, Nelson LM. Epidemiologic approaches to the study of Parkinson's disease etiology. Epidemiology 1999;10(3):327-36.

6. Barbeau A, Roy M, Bernier G, Campanella G, Paris S. Ecogenetics of Parkinson's disease: prevalence and environmental aspects in rural areas. Can J Neurol Sci 1987;14(1):36-41.

7. Butterfield PG, Valanis BG, Spencer PS, Lindeman CA, Nutt JG. Environmental antecedents of young-onset Parkinson's disease. Neurology 1993;43(6):1150-8.

8. Chan DK, Woo J, Ho SC, Pang CP, Law LK, Ng PW, Hung WT, Kwok T, Hui E, Orr K, Leung MF, Kay R. Genetic and environmental risk factors for Parkinson's disease in a Chinese population. J Neurol Neurosurg Psychiatry 1998;65(5):781-4.

9. De Michele G, Filla A, Volpe G, De Marco V, Gogliettino A, Ambrosio G, Marconi R, Castellano AE, Campanella G. Environmental and genetic risk factors in Parkinson's disease: a case- control study in southern Italy. Mov Disord 1996;11(1):17-23.

10. Fleming L, Mann JB, Bean J, Briggle T, Sanchez-Ramos JR. Parkinson's disease and brain levels of organochlorine pesticides. Ann Neurol 1994;36(1):100-3.

11. Golbe LI, Farrell TM, Davis PH. Follow-up study of early-life protective and risk factors in Parkinson's disease. Mov Disord 1990;5(1):66-70.

12. Gorell JM, Johnson CC, Rybicki BA, Peterson EL, Richardson RJ. The risk of Parkinson's disease with exposure to pesticides, farming, well water, and rural living. Neurology 1998;50(5):1346-50.

13. Herishanu YO, Kordysh E, Goldsmith JR. A case-referent study of extrapyramidal signs (preparkinsonism) in rural communities of Israel. Can J Neurol Sci 1998;25(2):127-33.

14. Hertzman C, Wiens M, Bowering D, Snow B, Calne D. Parkinson's disease: a case-control study of occupational and environmental risk factors. Am J Ind Med 1990;17(3):349-55.

15. Hertzman C, Wiens M, Snow B, Kelly S, Calne D. A case-control study of Parkinson's disease in a horticultural region of British Columbia. Mov Disord 1994;9(1):69-75.

16. Ho SC, Woo J, Lee CM. Epidemiologic study of Parkinson's disease in Hong Kong. Neurology 1989;39(10):1314-8.

17. Hubble JP, Cao T, Hassanein RE, Neuberger JS, Koller WC. Risk factors for Parkinson's disease. Neurology 1993;43(9):1693-7.

18. Liou HH, Tsai MC, Chen CJ, Jeng JS, Chang YC, Chen SY, Chen RC. Environmental risk factors and Parkinson's disease: a case-control study in Taiwan. Neurology 1997;48(6):1583-8.

19. Morano A, Jimenez-Jimenez FJ, Molina JA, Antolin MA. Risk-factors for Parkinson's disease: case-control study in the province of Caceres, Spain. Acta Neurol Scand 1994;89(3):164-70.

20. Ohlson CG, Hogstedt C. Parkinson's disease and occupational exposure to organic solvents, agricultural chemicals and mercury--a case-referent study. Scand J Work Environ Health 1981;7(4):252-6.

21. Rajput AH, Uitti RJ, Stern W, Laverty W, O'Donnell K, O'Donnell D, Yuen WK, Dua A. Geography, drinking water chemistry, pesticides and herbicides and the etiology of Parkinson's disease. Can J Neurol Sci 1987;14(3 Suppl):414-8.

22. Rocca WA, Anderson DW, Meneghini F, Grigoletto F, Morgante L, Reggio A, Savettieri G, Di Perri R. Occupation, education, and Parkinson's disease: a case-control study in an Italian population. Mov Disord 1996;11(2):201-6.

23. Seidler A, Hellenbrand W, Robra BP, Vieregge P, Nischan P, Joerg J, Oertel WH, Ulm G, Schneider E. Possible environmental, occupational, and other etiologic

factors for Parkinson's disease: a case-control study in Germany. Neurology 1996;46(5):1275-84.

24. Semchuk KM, Love EJ, Lee RG. Parkinson's disease and exposure to rural environmental factors: a population based case-control study. Can J Neurol Sci 1991;18(3):279-86.

25. Semchuk KM, Love EJ, Lee RG. Parkinson's disease and exposure to agricultural work and pesticide chemicals. Neurology 1992;42(7):1328-35.

26. Semchuk KM, Love EJ, Lee RG. Parkinson's disease: a test of the multifactorial etiologic hypothesis. Neurology 1993;43(6):1173-80.

27. Smargiassi A, Mutti A, De Rosa A, De Palma G, Negrotti A, Calzetti S. A case-control study of occupational and environmental risk factors for Parkinson's disease in the Emilia-Romagna region of Italy. Neurotoxicology 1998;19(4-5):709-12.

28. Stern M, Dulaney E, Gruber SB, Golbe L, Bergen M, Hurtig H, Gollomp S, Stolley P. The epidemiology of Parkinson's disease. A case-control study of young- onset and old-onset patients. Arch Neurol 1991;48(9):903-7.

29. Tanner CM, Chen B, Wang W, Peng M, Liu Z, Liang X, Kao LC, Gilley DW, Goetz CG, Schoenberg BS. Environmental factors and Parkinson's disease: a case-control study in China. Neurology 1989;39(5):660-4.

30. Wong GF, Gray CS, Hassanein RS, Koller WC. Environmental risk factors in siblings with Parkinson's disease. Arch Neurol 1991;48(3):287-9.

31. Checkoway H, Farin FM, Costa-Mallen P, Kirchner SC, Costa LG. Genetic polymorphisms in Parkinson's disease. Neurotoxicology 1998;19(4-5):635-43.

32. Gorrell JM, DiMonte D, Graham D. The role of the environment in Parkinson's disease. Environ Health Perspect 1996;104(6):652-4.

33. Landi MT, Ceroni M, Martignoni E, Bertazzi PA, Caporaso NE, Nappi G. Gene-environment interaction in parkinson's disease. The case of CYP2D6 gene polymorphism. Adv Neurol 1996;69:61-72.

34. Calne DB, Snow BJ, Lee C. Criteria for diagnosing Parkinson's disease. Ann Neurol 1992;32(Suppl):S125-7.

35. Rajput AH. Frequency and cause of Parkinson's disease. Can J Neurol Sci 1992;19(1 Suppl):103-7.

36. Bennett DA, Beckett LA, Murray AM, Shannon KM, Goetz CG, Pilgrim DM, Evans DA. Prevalence of parkinsonian signs and associated mortality in a community population of older people. N Engl J Med 1996;334(2):71-6.

37. de Rijk MC, Tzourio C, Breteler MM, Dartigues JF, Amaducci L, Lopez-Pousa S, Manubens-Bertran JM, Alperovitch A, Rocca WA. Prevalence of parkinsonism and Parkinson's disease in Europe: the EUROPARKINSON Collaborative Study. European Community Concerted Action on the Epidemiology of Parkinson's disease. J Neurol Neurosurg Psychiatry 1997;62(1):10-5.

38. Morgante L, Rocca WA, Di Rosa AE, De Domenico P, Grigoletto F, Meneghini F, Reggio A, Savettieri G, Castiglione MG, Patti F, et al. Prevalence of Parkinson's disease and other types of parkinsonism: a door-to-door survey in three Sicilian municipalities. The Sicilian Neuro-Epidemiologic Study (SNES) Group. Neurology 1992;42(10):1901-7.

39. Trenkwalder C, Schwarz J, Gebhard J, Ruland D, Trenkwalder P, Hense HW, Oertel WH. Starnberg trial on epidemiology of Parkinsonism and hypertension in the elderly. Prevalence of Parkinson's disease and related disorders assessed by a door-to-door survey of inhabitants older than 65 years. Arch Neurol 1995;52(10):1017-22.

40. Fahn S, Elton R, Members of the UPDRS Development Committee. Unified Parkinson's Disease Rating Scale. In: Fahn S, Marsden C, Calne D, Goldstein M, eds. Recent developments in Parkinson's disease. Vol. 2. Florham Park, NJ: Macmillan Health Care Information, 1987;153-163.

41. Thompson ML, Myers JE, Kriebel D. Prevalence odds ratio or prevalence ratio in the analysis of cross sectional data: what is to be done? Occup Environ Med 1998;55(4):272-7.

42. Morens DM, Grandinetti A, Reed D, White LR, Ross GW. Cigarette smoking and protection from Parkinson's disease: false association or etiologic clue? Neurology 1995;45(6):1041-51.

43. Gorell JM, Rybicki BA, Johnson CC, Peterson EL. Smoking and Parkinson's disease: a dose-response relationship. Neurology 1999;52(1):115-9.

44. Grandinetti A, Morens DM, Reed D, MacEachern D. Prospective study of cigarette smoking and the risk of developing idiopathic Parkinson's disease. Am J Epidemiol 1994;139(12):1129-38.

45. Hellenbrand W, Seidler A, Robra BP, Vieregge P, Oertel WH, Joerg J, Nischan P, Schneider E, Ulm G. Smoking and Parkinson's disease: a case-control study in Germany. Int J Epidemiol 1997;26(2):328-39.

46. Waite LM, Broe GA, Creasey H, Grayson D, Edelbrock D, O'Toole B. Neurological signs, aging, and the neurodegenerative syndromes. Arch Neurol 1996;53(6):498-502.

47. Ferraz HB, Bertolucci PH, Pereira JS, Lima JG, Andrade LA. Chronic exposure to the fungicide maneb may produce symptoms and signs of CNS manganese intoxication. Neurology 1988;38(4):550-3.

48. Semchuk KM, Love EJ. Effects of agricultural work and other proxy-derived case-control data on Parkinson's disease risk estimates. Am J Epidemiol 1995;141(8):747-54.

49. Koller W, Vetere-Overfield B, Gray C, Alexander C, Chin T, Dolezal J, Hassanein R, Tanner C. Environmental risk factors in Parkinson's disease. Neurology 1990;40(8):1218-21.

50. Kuopio AM, Marttila RJ, Helenius H, Rinne UK. Changing epidemiology of Parkinson's disease in southwestern Finland. Neurology 1999;52(2):302-8.

51. Hayes WJ, Laws ER. Handbook of pesticide toxicology. San Diego: Academic Press, 1991.

52. Bhatt MH, Elias MA, Mankodi AK. Acute and reversible parkinsonism due to organophosphate pesticide intoxication: five cases. Neurology 1999;52(7):1467-71.

53. Herishanu YO, Goldsmith JR. Clustering of Parkinson's disease in Southern Israel. An endemic Parkinsonism-impaired vibration sense syndrome. In: Nagatsu T, Fisher A, Yoshida M, eds. Basic, clinical, and therapeutic aspects of Alzheimer's and Parkinson's diseases. New York: Plenum Press, 1990;267-270.

54. Herishanu YO, Frisher S. Early parkinsonism in a rural population of Southern Israel (abstract). Mov Disord 1991;6:270.

55. Chiba K, Trevor A, Castagnoli N, Jr. Metabolism of the neurotoxic tertiary amine, MPTP, by brain monoamine oxidase. Biochem Biophys Res Commun 1984;120(2):574-8.

56. Simonian NA, Coyle JT. Oxidative stress in neurodegenerative diseases. Annu Rev Pharmacol Toxicol 1996;36:83-106.

57. Schapira AH, Gu M, Taanman JW, Tabrizi SJ, Seaton T, Cleeter M, Cooper JM. Mitochondria in the etiology and pathogenesis of Parkinson's disease. Ann Neurol 1998;44(3 Suppl 1):S89-98.

58. Howell N, Bindoff LA, McCullough DA, Kubacka I, Poulton J, Mackey D, Taylor L, Turnbull DM. Leber hereditary optic neuropathy: identification of the same mitochondrial ND1 mutation in six pedigrees. Am J Hum Genet 1991;49(5):939-50.

59. Mizuno Y, Yoshino H, Ikebe S, Hattori N, Kobayashi T, Shimoda-Matsubayashi S, Matsumine H, Kondo T. Mitochondrial dysfunction in Parkinson's disease. Ann Neurol 1998;44(3 Suppl 1):S99-109.

60. Kurth JH, Kurth MC, Poduslo SE, Schwankhaus JD. Association of a monoamine oxidase B allele with Parkinson's disease. Ann Neurol 1993;33(4):368-72.

61. Tobe VO, Taylor SL, Nickerson DA. Single-well genotyping of diallelic sequence variations by a two-color ELISA-based oligonucleotide ligation assay. Nucleic Acids Res 1996;24(19):3728-32.

62. Ho SL, Kapadi AL, Ramsden DB, Williams AC. An allelic association study of monoamine oxidase B in Parkinson's disease. Ann Neurol 1995;37(3):403-5.

63. Morimoto Y, Murayama N, Kuwano A, Kondo I, Yamashita Y, Mizuno Y. Association analysis of a polymorphism of the monoamine oxidase B gene with Parkinson's disease in a Japanese population. Am J Med Genet 1995;60(6):570-2.

64. Costa P, Checkoway H, Levy D, Smith-Weller T, Franklin GM, Swanson PD, Costa LG. Association of a polymorphism in intron 13 of the monoamine oxidase B gene with Parkinson disease. Am J Med Genet 1997;74(2):154-6.

65. Checkoway H, Franklin GM, Costa-Mallen P, Smith-Weller T, Dilley J, Swanson PD, Costa LG. A genetic polymorphism of MAO-B modifies the association of cigarette smoking and Parkinson's disease. Neurology 1998;50(5):1458-61.

66. Menegon A, Board PG, Blackburn AC, Mellick GD, Le Couteur DG. Parkinson's disease, pesticides, and glutathione transferase polymorphisms. Lancet 1998;352(9137):1344-6.

67. Checkoway H, Pearce N, Crawford-Brown DJ. Research methods in occupational epidemiology. New York: Oxford University Press, 1989.

68. Armstrong BK, White E, Saracci R. Principles of exposure measurement in epidemiology. Oxford ; New York: Oxford University Press, 1992.

69. Blair A, Zahm SH. Patterns of pesticide use among farmers: implications for epidemiologic research. Epidemiology 1993;4(1):55-62.

70. Baumgarten M, Siemiatycki J, Gibbs GW. Validity of work histories obtained by interview for epidemiologic purposes. Am J Epidemiol 1983;118(4):583-91.

71. Stewart WF, Tonascia JA, Matanoski GM. The validity of questionnaire-reported work history in live respondents. J Occup Med 1987;29(10):795-800.

72. Rosenberg CR, Mulvihill MN, Fischbein A, Blum S. An analysis of the validity of self reported occupational histories using a cohort of workers exposed to PCBs. Br J Ind Med 1987;44(10):702-10.

73. Rosenberg CR. An analysis of the reliability of self reported work histories from a cohort of workers exposed to polychlorinated biphenyls. Br J Ind Med 1993;50(9):822-6.

74. Bond GG, Bodner KM, Sobel W, Shellenberger RJ, Flores GH. Validation of work histories obtained from interviews. Am J Epidemiol 1988;128(2):343-51.

75. Bourbonnais R, Meyer F, Theriault G. Validity of self reported work history. Br J Ind Med 1988;45(1):29-32.

76. Brisson C, Vezina M, Bernard PM, Gingras S. Validity of occupational histories obtained by interview with female workers. Am J Ind Med 1991;19(4):523-30.

77. Holmes E, Garshick E. The reproducibility of the self-report of occupational exposure to asbestos and dust. J Occup Med 1991;33(2):134-8.

78. Joffe M. Validity of exposure data derived from a structured questionnaire. Am J Epidemiol 1992;135(5):564-70.

79. van der Gulden JW, Jansen IW, Verbeek AL, Kolk JJ. Repeatability of self-reported data on occupational exposure to particular compounds. Int J Epidemiol 1993;22(2):284-7.

80. Ferguson WL. Pesticide use on selected crops: aggregated data, 1977-80. Agriculture information bulletin; no. 494; pp. 2-10. U.S. Dept. of Agriculture Economic Research Service, 1985.

81. Rothman KJ, Greenland S. Modern epidemiology. 2nd ed. Philadelphia, PA: Lippincott-Raven, 1998.

CURRICULUM VITAE

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Educational Background

University of Washington, Seattle, WA
Ph.D., Epidemiology, 1999

University of Washington, Seattle, WA
M.S., Epidemiology, 1996

University of California, Berkeley, CA
B.S., Genetics, 1986; *magna cum laude*

Professional Positions

1998 (Dec.)	Epidemiology Instructor, Universidad Nacional, Heredia, Costa Rica
1996	Teaching Assistant; University of Washington, Department of Epidemiology, Seattle, WA
1995	Epidemiology Consultant/Programmer; Seattle Children's Home, Seattle, WA
1994 - 1996	Instructor (of Research Methods), Northwest Institute of Acupuncture and Oriental Medicine, Seattle, WA
1993 - 1999	Research Assistant; University of Washington, Departments of Epidemiology and Environmental Health, Seattle, WA
1991 - 1992	Teacher (half-time); Arrowsmith Academy, Berkeley, CA
1989 - 1993	Computer Programmer/Analyst, Project Leader; Shared Medical Systems Inc., Oakland, CA
1987 - 1988	Computer Programmer; Innovative Interfaces Inc., Berkeley, CA
1985 - 1986	Research Assistant; University of California, Department of Genetics (Population), Berkeley, CA
1983 - 1985	Research Assistant; University of California, Department of Genetics (Molecular), Berkeley, CA

1982 - 1987	Computer Programmer; Sterling Software Inc., Ames Research Center, Mountain View, CA
1981 - 1982	Computer Programmer; Lexidata Corp., Billerica, MA
1980	Computer Programmer; Conceptual Designs Software Corp., Framingham, MA

Scholarships, Fellowships, Awards

National Institute of Environmental Health Predoctoral Fellowship, 1994 - 1998

McDonald Scholarship, Kraft Scholarship, Wrass Scholarship, UC Berkeley Scholarship, University of California, Berkeley, 1982 - 1986

National Merit Scholarship Finalist, 1980

Publications

Engel LS, Keifer MC, Checkoway H, Robinson LR, Vaughan TL (1998): Neurophysiological function in farm workers exposed to organophosphate pesticides. Arch Environ Health 53:7-14

Presentations

Comparison of a traditional questionnaire with an icon/calendar-based questionnaire to assess occupational history; American Public Health Association Annual Meeting; Washington, DC; 1998

Test-retest reliability of an icon/calendar-based questionnaire used to assess occupational history; 4th International Symposium: Rural Health and Safety in a Changing World; Saskatoon, Saskatchewan, Canada; 1998

Comparison of a traditional questionnaire with an icon/calendar-based questionnaire to assess occupational history; Society of Epidemiologic Research 31st Annual Meeting; Chicago, Illinois; 1998

Comparison of a traditional questionnaire with an icon/calendar-based questionnaire to assess occupational history; 10th Annual UBC-UW Occupational and Environmental Health Conference; Blaine, Washington; 1998

Neurophysiological function in farm workers exposed to organophosphate pesticides; American Public Health Association Annual Meeting; New York, New York; 1996

Maternal occupation in agriculture and risk of adverse birth outcomes in Washington State; Society of Epidemiologic Research 28th Annual Meeting; Snowbird, Utah; 1995

Languages

Spanish