Title
Exposure Assessment of Pesticides and the Effect of Combinations of Pesticides on Parkinson's Disease

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Publication Date
2012

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA

Los Angeles

Exposure Assessment of Pesticides and the Effect of Combinations of Pesticides on Parkinson's Disease

A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy

in Epidemiology

by

Anthony Weirehn Wang

2012

ABSTRACT OF THE DISSERTATION

Exposure Assessment of Pesticides and

the Effect of Combinations of Pesticides on Parkinson's Disease

by

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Doctor of Philosophy in Epidemiology

University of California, Los Angeles, 2012

Professor Beate Ritz, Chair

Due to the heavy and expanding agricultural use of neurotoxic pesticides suspected to affect dopaminergic neurons, it is imperative to closely examine the role of pesticides in the development of Parkinson's disease (PD). We recruited 357 incident PD cases and 752 population-based controls from 2000-2010 in the Central Valley of California and collected demographic, covariate, as well as residential and occupational address information. We utilized a geographic information system (GIS)-based exposure assessment tool to estimate historical ambient exposure to agricultural pesticides at residential and occupational addresses.

Combined exposure to ziram, maneb, and paraquat at workplaces increased risk of PD three-fold and combined exposure to ziram and paraquat, excluding maneb exposure, was associated with an 80% increase in risk. Risk estimates for ambient workplace exposure were

greater than exposures at residences and were especially high for younger onset PD patients and when exposed in both locations.

We estimated a greater than two-fold increase in risk of developing PD for participants exposed to organophosphates, organochlorines, dithiocarbamates, and paraquat individually after adjusting for covariates. However after adjusting for other pesticides, only ambient exposure to organophosphates remained strongly associated, suggesting that pesticides from other classes have a high degree of co-exposure and may require combined exposure to affect PD risk. Longer duration of exposure and co-exposure to a large number of pesticides within the same year were both associated with strong increases in PD risk.

Ambient exposure to each organophosphate separately increased the risk of developing PD. However, it is difficult to estimate the risk associated with an individual pesticide due to the likelihood that participants were exposed to combinations of these pesticides rather than any one single pesticide. Combinations of organophosphates with mitochondrial disrupting properties exhibited larger risk increases and exposure-response patterns were observed with exposure to an increasing number of these chemicals.

Taken together, our results provide support that ambient co-exposure to pesticides contributes to the etiology of PD.

The dissertation of Anthony Weirehn Wang is approved.

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Jeff Bronstein

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2012

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Abbreviations

ACh	Acetylcholine
ALDH	Aldehyde dehydrogenases
ATP	Adenosine triphosphate
BBB	Blood brain barrier
CA DPR	California Department of Pesticide Regulation
CDWR	California Department of Water Resources
CGEP	UCLA Center for Gene and Environment in Parkinson's Disease
COMT	Catechol-O-methyl transferase
DA	Dopamine
DAT	Dopamine transporter
DTC	Dithiocarbamate
DOPAL	3,4-dihydroxyphenylacetaldehyde
GDS	Geriatric Depression Scale
GIS	Geographic information system
GPS	Global positioning system
GSH	Glutathione
GSSG	Oxidized glutathione
HRQoL	Health related quality of life
JEM	Job exposure matrix
LB	Lewy bodies
LD_{50}	Median lethal dose
LN	Lewy neurites
LU	Land use
MAO	Monoamine oxidase
MB	Maneb
MMSE	Mini Mental State Exam
Mn-EBDC	Ethylene-bis-dithiocarbamate
MPP^+	1-methyl-4-phenylpryidinium ion
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NADH	Nicotinamide adenine dinucleotide
OC	Organochlorine
OP	Organophosphate
OR	Odds ratio
PEG	Parkinson's, Environment and Gene study
PD	Parkinson's disease
PQ	Paraquat
PUR	Pesticide use reporting
ROS	Reactive oxygen species
RR	Risk ratio
SN	Substantia nigra
SNc	Substantia nigra pars compacta
TH	Tyrosine hydroxylase
UPDRS	United Parkinson's Disease Rating Scale
UPS	Ubiquitin-proteasome system
VMAT	Vesicular monoamine transporter
VMAT2	Vesicular monoamine transporter
Zi	Ziram

ACKNOWLEDGEMENTS

I would like to give my deepest gratitude to my mentor and chair of my dissertation committee, Dr. Beate Ritz, Professor of Epidemiology, Environmental Health Sciences and Neurology at the UCLA Schools of Public Health and Medicine. Dr. Ritz has been a consistent source of knowledge, advice, motivation, and support. This work was only possible through the dedication and work of the study participants and neurologists who gave so much of their time to the PEG and CGEP studies. I would like to thank our study staff for the role they played in participant recruitment, data collection, cleaning, and management. I would like to acknowledge the advice, support, and contributions to my papers I received from Drs. Nicole Gatto, Sadie Costello, and Shannon Rhodes. Dr. Myles Cockburn and his team in the Preventive Medicine Department at the University of California contributed immensely to this work by providing our GRAPES model, as well as estimating and processing the pesticide estimates. Drs. Onyebuchi Arah and Fei Yu also contributed to the analyses of this dissertation. I would also like to recognize Drs. Beate Ritz, Leeka Kheifets, Onyebuchi A. Arah, and Jeff Bronstein for providing valuable advice and serving on my dissertation committee.

Finally I would like to acknowledge my wife, Elana, my entire family, and friends for offering so much support, prayer, and encouragement throughout the development of this dissertation.

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"Ambient Exposure to Maneb, Ziram and Paraquat at Residential and Occupational Addresses and Parkinson's Disease in California's Central Valley" Anthony Wang, Sadie Costello, Myles Cockburn, Xinbo Zhang, Jeff Bronstein, Beate Ritz. Spotlight Session Presentation at Society for Epidemiologic Research Annual Meeting in Seattle, Washington, USA, June 21-24, 2010.

Chapter I: Introductory Background of PD:

Parkinson's disease (PD) is an idiopathic, chronic, progressive, neurodegenerative disease associated with aging.[1] The four cardinal symptoms of PD are 1) bradykinesia, 2) rigidity, 3) tremor at rest, and 4) loss of postural reflexes.[2,3] PD is the most common movement disorder associated with aging[3] and the second most common neurodegenerative movement disorder after Alzheimer's disease.[4] The annual incidence for PD ranges from 10 to 17 per 100,000 worldwide[5] and its prevalence for those over age 50 in 10 of the most populous nations is between 4.1 and 4.6 million in 2005 and will double to between 8.7 and 9.3 million by 2030.[6] The variance in the estimates of the incidence and prevalence of PD reflects the difficulty in diagnosing the disease based on clinical criteria, due to the lack of a definitive test .[2] Currently, diagnosis of parkinsonism requires the presence of at least two of the cardinal symptoms of PD. In order to make a diagnosis of PD, the causes of secondary parkinsonism must be excluded and even then a diagnosis can only be confirmed by post-mortem examination.[4] A clinicopathological study found that 82% of those clinically diagnosed with PD were confirmed to have PD at autopsy, emphasizing the need to improve the clinical diagnostic accuracy of PD using stringent criteria.[7,8]

Besides the four cardinal symptoms of PD, patients may also suffer other non-motor comorbidities including, but are not limited to, depression, dementia, injury from falls, sleep disruption, back problems, arthritis, and hypertension.[9-12] Limitations conferred by the symptoms and comorbidities of PD impose a substantial burden on the health related quality of life (HRQoL) of PD patients and will worsen as the patient ages.[11-13] Due to the physical limitations of PD patients they often require the constant attention of caregivers to assist them in their

activities of daily life. As caregivers adjust to changing family dynamics, physical and emotional stress, and increasing financial responsibilities they experience an increase in depression and overall decrease in their own HRQoL.[14]

Beyond the burdens placed upon PD patients and their caregivers, the economic burden of the costs associated with PD and its comorbidities is staggering. Direct health care cost of PD in the United States was estimated at $10,349 per patient per year and total costs may be as high as $23 billion annually.[15] The largest portions of this burden are the indirect costs of productivity loss of PD patients and the provision of uncompensated care by household caretakers.[15]

1.1. Pathogenesis of PD

The symptoms of PD are intimately connected with the dopaminergic system. Dopamine (DA) is a neurotransmitter involved in cognition, motor activity, reward, mood, attention, and learning.[16] The precursor of DA is L-tyrosine, which is converted to L-dopa by tyrosine hydroxylase (TH). L-dopa is then decarboxylated to DA by aromatic L-amino acid decarboxylase and packed into vesicles by the vesicular monoamine transporter (VMAT) before being released from the cell into the synapse. Once DA is released into the synaptic cleft and activates the receptors of the postsynaptic cell, dopamine transporter (DAT) will recycle the DA and transport it back into the presynaptic neuron where it will be metabolized by catechol-O-methyl transferase (COMT) and monoamine oxidase (MAO).[17] DA that has not been metabolized will be repackaged into VMAT.

1.2. Protective and Risk Factors for PD

Thus far, the cause of PD is unknown, but its etiology is thought to be multifactorial with aging, environmental factors, oxidative stress, and genetic factors contributing to the disease.[4] Age is the strongest risk factor for PD and incidence in men increases with age.[18,19] Men are also more likely than women to have PD and the male:female ratio seems to possibly increase with age, indicating that gender is an important risk factor.[19] It has also been found that PD rates among Whites are higher than Blacks and Asians.[19] Among the environmental risk factors of PD, tobacco use is the one most widely studied. Recently, a pooled analysis confirmed the findings of previous studies that found smoking to be a protective factor for PD.[20] Other environmental risk factors include coffee consumption and dietary factors.[4] Genetics are also thought to play a role in the etiology of early onset PD, however Mendelian type genetic factors do not appear to play a prominent role for the majority of those with late onset PD.[21] Genetic factors are generally thought to contribute to PD primarily through susceptibility genes coupled with environmental risk factors such as pesticide exposure.[4]

1.3. Study Objectives

This dissertation seeks to contribute toward addressing the association between exposures to combinations of pesticides and the risk of developing PD by utilizing geographic information system (GIS) based models.

1.3.1. Parkinson's Disease Risk from Ambient Exposure to Maneb, Ziram, and Paraquat

A previous study showed that combined exposure to maneb and paraquat confers a greater risk of PD than either pesticide alone.[22] It is important to assess ziram's contribution to PD risk because like maneb, it is a dithiocarbamate, and it also has been shown in studies to

damage the dopaminergic system. The first aim of this study is to determine if ziram with or without paraquat co-exposure, is implicated in PD etiology.

Like maneb, we expect combined exposure to ziram and paraquat to be positively associated with PD. We also expect exposure to ziram alone to have a weaker association with PD. Our specific aims are to:

1) Attempt to replicate our previous findings that combined exposure to maneb and paraquat is associated with a greater increase in the risk of PD than either pesticide alone, using the combined occupational and residential pesticide exposure estimates

2) Explore the association between ambient combined exposure to ziram, maneb, and paraquat with risk of developing PD at workplaces and residences

3) Explore the relationship between combined ambient exposure to ziram and paraquat as well as maneb and paraquat with risk of developing PD by age group

1.3.2. The Influence of Ambient Exposure to Paraquat, Dithiocarbamate, Organochlorine, and Organophosphate Pesticides on Parkinson's Disease Risk

Paraquat, dithiocarbamate, organochlorine, and organophosphate pesticides have all been shown to exhibit neurotoxic mechanisms that may destroy dopaminergic neurons in animal models and cell cultures. However, assessing these pesticides individually may not be sufficient due to the complex nature of pesticide exposure in human populations. There is often a significant amount of pesticide co-exposure due to successive applications of different pesticides and mixtures of pesticides used in agricultural applications. Thus, it is important to be aware of co-exposures to other pesticides when analyzing pesticide exposure data and interpreting the

results. Duration of pesticide exposure may also be important factors when determining risk of developing PD. The second aim of this study is to explore the relationships between paraquat and the dithiocarbamate, organochlorine, and organophosphate pesticide classes and PD risk. We also aim to determine if duration and intensity of pesticide co-exposure impact PD risk.

We expect a positive association between the pesticides and PD and an attenuation of these associations when adjusting for other pesticide exposure due to co-linearity of the pesticide exposures. We also expect that increasing duration and intensity of pesticide exposure will be associated with elevated PD risks. Our specific aims are to:

1) Explore the associations between paraquat, dithiocarbamates, organophosphates, and organochlorines with PD risk at workplaces and residences.

2) Examine if the risk of PD is greater when exposed to an increasing duration of pesticide exposure.

3) Assess the risk of PD associated with 'high intensity' pesticide exposure as defined by being exposed to more than 5 pesticides within the same year.

1.3.3. The Association Between Ambient Exposure to Organophosphates and Parkinson's Disease Risk

Organophosphate pesticides are the most commonly used pesticides in the world. However, few studies have studied the effect that specific organophosphate pesticides have on PD risk. The third aim of this study is to assess if specific organophosphates affect PD risk and whether organophosphates with certain presumed neurotoxic mechanisms, such as disrupting mitochondrial function, contribute more strongly to PD risk.

We hypothesize that exposures to organophosphate pesticides will be generally associated with increased PD risk. Our specific aims are to:

1) Explore the association between specific organophosphate and PD risk.

2) Assess the relationship between organophosphates with specific functions attributed to them such as being acutely toxic, teratogenicity, endocrine disruption, carcinogenicity, or mitochondrial disruption and PD risk.

3) Determine if exposure to an increasing number of organophosphates contributes to greater risk of developing PD.

Chapter II: Literature Reviews

2.1. Pathology

PD is characterized by loss of dopaminergic substantia nigra (SN) neurons, resulting in a depletion of striatal dopamine. As PD progresses and dopaminergic neurons die, distinctive inclusion bodies can be found in the surviving dopaminergic neurons. [3,23] These inclusion bodies include Lewy neurites (LN) and Lewy bodies (LB).[24,25] The pattern of LB and LN accumulation can be used to distinguish PD patients from those suffering from other neurodegenerative diseases (i.e. LB and LN in PD patients can be found in the substantia nigra, locus cereleus, hypothalamus, nucleus basalis, cranial nerve motor nuclei, cerebral cortex, and the central and peripheral divisions of the autonomic nervous system, whereas inclusion bodies in the amygdala is a hallmark of Alzheimer's disease).[23,24,26] Alpha-synuclein, a major component of inclusion bodies, is a protein normally found in the axons and presynaptic boutons of neurons. In the neurons of those with PD, alpha-synuclein proteins undergo a conformational change to a β-sheet

structure causing an aggregation of misfolded alpha-synuclein molecules.[23] This accumulation of alpha-synuclein often coupled with neuroinflammation leads to neurodegeneration and is thus hypothesized to play a causal role in PD.[23,27,28]

This build up of alpha-synuclein implicates that the inhibition of the ubiquitin-proteasome system (UPS) is a factor in the etiology of PD.[29] The UPS degrades mutated, misfolded, denatured, misplaced, or damaged proteins by labeling these unwanted proteins with ubiquitin molecules, transporting the ubiquitinated proteins into the proteasome, and then degrading the proteins into amino acids by proteasome regulators to be recycled into new proteins.[30,31] Since UPS processes are ATP-dependent, mitochondrial dysfunction may contribute to proteasomal inhibition that leads to an accumulation of alpha-synuclein, a hallmark of PD.[32] In fact, the enzymatic activities of proteasome are impaired in the SN in those with idiopathic PD.[29]

SN neurons are more susceptible to cellular damage because they use DA as their main neurotransmitter, an unstable molecule that is easily oxidized to form reactive oxygen species (ROS).[33] The presence of ROS and a loss of reducing substances, such as glutathione, can cause the peroxidation of lipids that can destroy cell membranes and neurons, which is referred to as oxidative stress.[34,35] The brain is particularly vulnerable to oxidative stress because it is rich in polyunsaturated fatty acids, which are prone to peroxidation.[36] When DA is not stored in vesicles, its oxidation produces a large amount of ROS that leads to oxidative stress and cell death.[37] For example, it is speculated that if dopamine catabolism decreases due to an alteration in COMT, reactive oxidative forms of dopamine could increase oxidative damage in the striatum.[17] PD cases also exhibit a reduction of VMAT within the striatum, which may contribute to the accumulation of DA outside of vesicles and oxidative stress.[38]

Oxidative stress also damages mitochondrial membranes, causing the electron transport chain to dysfunction.[39] Electron transport chain impairment then leads to ATP loss, a decrease in the production of the antioxidant, glutathione (GSH), additional oxidative stress, and more damage to the mitochondria. Energy deficiency caused by impaired mitochondrial function may lead to neuronal cell death through the activation of apoptotic cascades.[40]

2.2. Pesticide and PD

2.2.1. Overview of Past Studies

An ecologic study conducted in Quebec in the 1980s found that the prevalence of PD was higher in rural areas with a higher prevalence in agricultural areas.[41] This finding, coupled with the discovery that the herbicide, paraquat, is structurally similar to the neurotoxin, 1-methyl-4-phenylpryidinium ion (MPP^+), which has been shown to cause parkinsonism in those exposed to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), its precursor[42] has led researchers to investigate rural environmental factors that may play a role in the etiology of PD. A case-control study in China, found that risk of PD was increased in those who worked in industrial chemical plants that processed pesticides, but did not find factors of rural living to be associated with PD.[43] This finding is consistent with the hypothesis that pesticides are a risk factor for PD because at the time the study was conducted, synthetic pesticides were not available to farmers in rural villages, which may explain the protective effect of rural living for PD in this Chinese community. [43]

Although case-control studies are ideal to study rare diseases, widely conflicting results have been published with studies reporting odds ratios ranging from 0.5 to 7.0.[44-47] Several methodological limitations contributed to these conflicting results, including difficulties in

ascertaining PD case status, inappropriate control selection methods, variations in pesticide exposure definitions, lack of statistical power to detect associations between pesticides and PD, and self-reported pesticide exposure. Since there is no definitive test to diagnose PD prior to an autopsy allowing the study of brain pathology, diagnosis mainly relies on clinical symptoms. Diagnosis of PD based on these criteria depend on the person making the diagnosis, which may vary between studies, causing these studies to be prone to varying degrees of disease misclassification. Biased methods of control selection used by studies may have affected their ability to detect associations between pesticides and PD due to selecting controls with similar exposure patterns as cases.[48] Taylor et al.[49] asked their PD cases to identify friends and family members to participate in their study as controls. This selection of controls could potentially be associated with factors related to pesticide exposure such as rural living and farming, biasing any association between pesticide exposure and PD towards the null. Another study that employed friend controls asked cases to nominate three non-parkinsonism subjects to participate as controls, reported that 87.2% of cases and 91.3% of controls were ever exposed to any type of insecticides and thus did not detect an association between insecticides and PD.[44] Yet, another study matched on socio-cultural factors and included controls that lived in the same area as cases.[50] Matching on such potential risk factors that may affect exposure will make the exposure prevalence of non-cases more similar to that of cases, and is known as over-matching that biases effect estimates towards the null.[51] Deficiencies and differences in exposure definition can be a source of variation in effect estimates reported by studies. Studies that analyzed general pesticide data (i.e. ever/never exposed to pesticides, insecticides, or herbicides) may include pesticides with biological mechanisms that may increase the risk of PD as well as pesticides that do not have such mechanisms, masking any associations that specific pesticides may have with

PD.[45,50,52] Another methodological limitation in general is that pesticides are often applied either in conjunction with each other or in sequence (once a pest becomes immune to one type of pesticide), thus human occupational exposure to pesticides usually is a mixture.[53] Thus attempts to detect associations between specific pesticides or chemical classes with PD will be difficult due to collinearity between pesticides. Many of these studies also suffer from a paucity of exposed cases, reducing their power to detect associations between specific pesticides and risk of PD. These factors may reduce a study's ability to detect associations between specific pesticides and PD.[54] Finally, the vast majority of case-control studies relied on subjects to self-report their pesticide exposure over long periods of time. This is extremely problematic as cases may be more motivated to identify past exposures and this may lead to information bias.[55] Study subjects also may not be able to recall usage of specific pesticides leading to significant non-differential exposure misclassification, likely biasing the association between pesticide exposure PD towards the null.[48]

There have been a few cohorts that have allowed researchers to explore the links between pesticides and PD, including the Cancer Prevention Study II Nutritional Cohort,[56] PAQUID study,[57] Honolulu Heart Program,[58] Agricultural Health Study,[59] and a cohort of orchardists in Washington state,[60] the last two being occupational cohorts. Kamel et al. conducted a nested case-control study of licensed pesticide applicators and their spouses within the Agricultural Health Study and identified 78 incident PD cases.[59] Incident PD cases were defined by self-reported PD during the follow-up interview if they did not report PD at enrollment into the study five years earlier. It was difficult to be certain if being ever exposed to pesticides was associated with increased risk of incident PD (OR=1.3; 95% CI=0.5,3.3) while there appears to be a dose-response relationship between cumulative lifetime days of pesticide use and risk of PD (Highest

category, >=397 days vs. lowest category: OR=2.3; 95% CI=1.2,4.5; p for trend=0.009). These seemingly conflicting results can be attributed to the fact that the vast majority of the cases and controls were exposed to pesticides (91% of the cases and 83% of the controls) and thus odd ratios would be biased towards no effect. Kamel et al. also presented an analysis of 47 specific pesticide agents, finding only statistically significant associations with two herbicides and increased risk of incident PD and only 3 herbicides, 2 fungicides, and 1 fumigant associated with at least a 50% increase in the risk of incident PD. Difficulties in detection associations between PD and specific pesticide compounds may be attributed to the paucity of PD cases available for analysis and also due to the possibility that multiple pesticides must act on the dopaminergic system in order to increase risk of PD. Engel et al. also presented results for the associations between pesticide and risk of PD and found that there were no specific pesticides or group of pesticides that were associated with an increased risk.[60] However, misclassification of case status may have introduced significant bias into the study since only one PD case was diagnosed by a physician and the other cases (n=65) were only diagnosed as parkinsonism by a nurse trained in neurological examinations. Furthermore, exposure misclassification is also likely to have contributed to these null findings, since researchers relied on subject recall of historical use of specific pesticides.

The non-occupational cohorts studied general exposure to pesticides at workplaces but also suffered from small numbers of exposed cases. Petrovitch et al. identified 116 cases among plantation workers in Hawaii.[58] Workers who had worked for more than 10 years on sugarcane plantations were found to be at in increased risk of developing PD (RR=2.1; 95% CI=0.9,5.1). However, self-reported pesticide exposure was not significantly associated with PD. The authors suggested that a small sample size and exposure misclassification from subject recall may have

led to the null results of pesticide exposure and risk of PD. A cohort study of elderly French subjects identified 24 incident cases of PD and a cumulative pesticide exposure index was calculated from a job exposure matrix (JEM) created by a panel of six occupational classification experts.[57] Only pesticide exposure in men was found to be associated with PD, with occupationally exposed men having a 5-fold increase in the risk of PD compared with unexposed men (RR=5.6; 95% CI=1.5,21.6). Another study conducted among male French health insurance enrollees found PD to be associated with organochlorine (OR=2.4; 95%CI=1.2-5.0) and organophosphate (OR=1.8; 95%CI=0.9-3.7) insecticides.[61] Finally, Ascherio et al. conducted a cohort study within the Cancer Prevention Study and were able to identify 413 cases out of 142,912 total cohort members and found that farmers exposed to pesticides had similar risk of PD (RR=1.6; 95%CI=0.9,2.7) compared to non-farmers also exposed to pesticides (RR=1.7; 95%CI=1.2,2.5).[56]

2.2.2. Organophosphates

Organophosphates are the most commonly applied insecticides worldwide[62]. Organophosphates can be absorbed by inhalation, ingestion, and skin penetration and can also be highly toxic with a LD_{50} of up to 3-8mg/kg[63]. Parkinsonism in humans caused by organophosphate exposure was reported by Bhatt et al.[64] Organophosphate exposure occurred through ingestion in one patient, household fumigation in two patients, and entry into a previously fumigated home in two other patients. These patients developed the cardinal signs of parkinsonism that varied in severity depending on the amount of organophosphate pesticide they were exposed to. However, treatment with Levodopa did not appear to improve any of the patients' clinical symptoms. There have been a few studies that have reported conflicting epidemiologic evidence of an association between organophosphate exposure and PD, however

all studies suffered from low statistical power due to only having a few exposed cases. Hancock et al. conducted a family-based case-control study and found that self-reported organophosphate exposure was associated with PD (OR=1.89; 95% CI=1.11,3.25).[65] Firestone et al. reported no association between organophosphate exposure and PD (OR=1.07; 95% CI=0.46,2.49)[66]. Another case-control study conducted in a horticultural region of British Columbia reported inconclusive results about the relationship between organophosphate exposure and PD (OR=1.23; 95% CI=0.62,2.45).[67]

Research on animal models has proposed a few mechanisms of how organophosphate pesticides may act to potentially contribute to PD. Organophosphates are known to be potent achetylcholinesterase (AChE) inhibitors and readily cross the blood-brain barrier.[68] Acetylcholine (ACh), unlike other neurotransmitters, can only be degraded by AChE via hydrolysis and the resulting product is then transported back into the presynaptic terminal.[62] Thus, the inhibition of AChE causes an accumulation of ACh at cholinergic synapses causing cells to dysfunction by increasing its ATP consumption while disrupting oxidative phosphorylation, leading to the production of ROS.[62,69-71] Since all organophosphates act by inhibiting AChE by phosphorylating it, repeated exposure to the same or different organophosphates may lead to significant additive toxicity.[63,72] Organophosphates have also been suggested to cause mitochondrial dysfunction by inhibiting mitochondrial complexes I through IV, causing cell damage and death.[73] The inhibition of the electron transport chain causes an accumulation of electron carrier proteins in its reduced form and unused oxygen, leading to the conversion of oxygen into ROS.[74] GSH also acts as an antioxidant by reducing lipid peroxides and as a result is converted to oxidized glutathione (GSSG).[75] Rat brains that are exposed to organophosphates, exhibit a reduction in GSH levels, leading to further oxidative

damage.[36,75,76] Thus besides inhibiting acetylcholinesterase, organophosphates disturb redox processes that inhibit antioxidant enzymes thus enhancing lipid peroxidation and oxidative stress.

2.2.3. Organochlorines

Organochlorines are common active ingredients in home garden products, agricultural, structural, and environmental pesticides and can be absorbed through ingestion, inhalation, and skin penetration.[63] Since organochlorines persist in the environment for long periods of time, significant exposure may result from swallowing aerosol or dust particles laden with organochlorine pesticides.[63,77] Organochlorines have long been implicated in the etiology of PD because of post-mortem studies that found brains of PD patients to contain detectable traces of organochlorines more often than those of people who had died of other illnesses.[78,79] Epidemiological studies have reported conflicting results regarding the association between organochlorine exposure and PD. Hancock et al. reported an increased risk of PD among patients recruited from hospital, physical referrals, and self-referrals who were ever exposed to organochlorine pesticides (OR=1.99; 95%CI=1.09-3.64).[65] Similarly, a case-control study conducted in Germany found that ever being exposed to organochlorines was positively associated with increased risk of PD for neurologic clinic patients compared to regional control subjects.[55] A small case-control study, with only 16 cases exposed organochlorines, conducted in Canada was unable to detect an association between handling or working in areas recently sprayed with organochlorine pesticides and the risk of PD (OR=0.89;95%CI=0.45,1.76).[67] Finally, a cohort study conducted amongst orchardists working in Washington state also reported no association between ever exposure to organochlorines and increased risk of PD (PR=0.8;95%CI=0.5-1.3).[60]

Organochlorines have been shown to target the dopaminergic system in animal models. Doves that were fed low doses of dieldrin for 8 weeks exhibited reductions of dopamine by 58.6% compared to doves fed control diets.[80] Sanchez-Ramos et al. showed that dopaminergic neurons may be especially vulnerable to dieldrin, a potent organochlorine, as less of it is required to destroy dopaminergic neurons compared to nondopminergic neurons.[81] Several animal studies have proposed mechanisms that may contribute to the neurotoxicity of organochlorines. Heptachlor has been shown to increase DAT and VMAT2 expression, however it also reduces the uptake of DA into vesicles by 45% in mice, leading to an accumulation of DA within the cell, causing oxidative stress.[82] Dieledrin was reported to decrease the levels of striatal GSH, which facilitates oxidative stress.[83] Dieledrin also targets the mitochondria by inhibiting oxidative phosphorylation near complex III, reducing cellular ATP production and increases ROS levels.[84] ROS has been shown to induce the mitochondria to release cytochrome c into the cytoplasm, which signals the apoptotic caspase cascade.[77,85]

2.2.4. Paraquat

Paraquat is a non-selective contact herbicide that is widely used in agriculture for control of weeds.[63,86] Paraquat primarily targets the lung through the generation of free radicals that cause oxidative damage to lung tissues.[87] However, damage to the brain has also been observed in those who have died of paraquat poisoning.[88,89] Paraquat was hypothesized to be involved in the etiology of PD due to its structural similarities with MPP^+.[90] MPP^+ is the toxic metabolite of MPTP that induces parkinsonism in humans and primates exposed to it. MPTP was first implicated to be involved in PD when persons developed parkinsonism after injecting synthetic heroin containing MPTP.[42] In order to destroy dopaminergic neurons in the substantia nigra pars compacta (SNc), MPTP must be converted into MPP^+ intracerebrally by monoamine oxidase B

because MPP^+ cannot cross the blood-brain barrier (BBB).[86] From these findings, much research has been conducted to assess the neurotoxicity of paraquat in animal models, its ability to breach the BBB, and evidence of its association with PD in humans. The only human study to date with adequate power to detect an association between paraquat exposure and PD was conducted by Liou et al. in Taiwan.[91] They recruited 120 PD cases and matched 240 hospital controls on age (+/- 2 years) and sex. Paraquat is commonly applied on rice patties in Taiwan and both paraquat exposure (OR= 3.22; 95% CI= 2.41-4.31) and rice farming (OR=1.70; 95%CI= 1.13,2.58) were associated with an increased risk of PD.

Paraquat's deleterious effects on the dopaminergic system have been well documented by the majority of studies that have explored this association in animal models.[92,93] Studies reported a loss of SN neurons and decrease of striatal dopamine following internigral injection of paraquat, indicating that paraquat induces neuronal degeneration.[90,94] The mechanism behind paraquat's neurotoxicity is thought be its ability to increase the production of oxygen free radicals by either the redox cycling reactions of paraquat with molecular oxygen and/or an NADH-dependent formation of superoxide anions.[90] Through these mechanisms, paraquat may selectively target dopaminergic neurons, due the particular vulnerability of nigrostriatal neurons to oxidative stress.[34,90] Further implicating paraquat in PD is that mice injected with paraquat has been shown to cause an up-regulation in the production of alpha-synuclein in the SN.[95] In animal models, researchers have demonstrated that paraquat is also able penetrate the BBB, possibly via Na^+-dependent neutral amino acid transport system expressed in the brain capillaries.[86] The mechanism of how paraquat is taken up into dopaminergic terminals is unclear. Researchers have postulated that paraquat uptake is mediated by DAT since inhibiting DAT reduces paraquat uptake and protects cells against paraquat toxicity.[96,97] However, another study found that

paraquat elicits dopaminergic neurotoxicity independent of DAT expression and paraquat did not significantly inhibit dopamine uptake via DAT, thus the researchers concluded that paraquat uptake is not mediated by DAT.[98] Once inside the cell, paraquat can accept an electron from the mitochondrial complex I, inhibiting mitochondrial respiration and producing a paraquat radical that increases lipid peroxidation and damages dopaminergic neurons.[99] Paraquat also causes an increase in extracellular glutamate, either by stimulating striatal neurons to release glutamate or by inhibiting its reuptake, that then leads to a excitotoxic cascade that damages dopaminergic terminals.[96] Furthermore, long-term exposure to paraquat has been shown to induce a toxic effect on the mesocortical dopaminergic pathway and is a long-lasting process that may resemble pathological degenerative mechanisms of PD.[100]

2.2.5. Dithiocarbamates

Dithiocarbamates are widely used fungicides that are formulated as wettable powders and exposure to these pesticides have been known to cause neural disturbances.[63] Although dithiocarbamates are considered to have low toxicity, chronic human exposure to maneb has been linked to the development of parkinsonism.[101] A study conducted amongst 50 rural workers exposed to maneb reported higher prevalence of plastic rigidity, cogwheel phenomenon, headache, fatigue, memory complaints, postural tremor, and bradykinesia when compared to 19 rural workers without exposure.[102] A man with chronic exposure to maneb developed permanent parkinsonism two years after his last reported exposure.[103] Another man who sprayed maneb and zineb on a cucumber fields twice a week reported a loss of consciousness, convulsions, and right hemiparesis.[104]

Although human data connecting dithiocarbamates to PD is sparse, animal models suggest that these pesticides may have mechanisms that contribute to the etiology of PD. Maneb's active ingredient, ethylene-bis-dithiocarbamate (Mn-EBDC), causes dopaminergic degeneration when delivered to the lateral ventricle of the rat.[101] Significant efflux of vesicular DA occurred when Mn-EBDC was given to rats, increasing oxidative stress.[101] Mn-EBDC has also been shown to inhibit mitochondrial complex III, reducing ATP production and contributing to cell death.[101] These mechanisms of Mn-EBDC also lead to disruption of proteasome function, causing an accumulation of alpha-synuclein and other damaged proteins.[105] Ziram also acts on the UPS system by reducing E1 ligase activity, inhibiting the activation of ubiquitin and abnormal protein degradation.[106] The metabolites of ziram may also inhibit aldehyde dehydrogenases (ALDH), which is involved in the conversion of 3,4-dihydroxyphenylacetaldehyde (DOPAL), a dopamine metabolite that is toxic to dopaminergic neurons.[107-109]

2.2.6. Multi-hit Hypothesis

The majority of epidemiological studies attempting to test an association between PD and pesticides define pesticide exposure as non-specific categories such as "ever exposed to any pesticide" or "ever exposed to herbicides", a flawed approach in many senses. First, animal studies have demonstrated that certain pesticides have specific mechanisms that act on different elements of the dopaminergic system to cause PD. Studies that either do not identify exposure to specific pesticides or combinations of pesticides have reported conflicting findings because it is not certain if the actual pesticides their subjects are exposure to have biological mechanisms that contribute to the etiology of PD.[46,49,57,110-114] Second, the intensity or level of exposure to pesticides is also important in determining if the exposure is adequate to cause PD. An

individual that is exposed to low levels of a pesticide known to cause PD may be able to metabolize the pesticide before it is able to affect its target sites. Studies that do not estimate cumulative or intensity of pesticide exposure may experience significant heterogeneity within their exposure categories as highly exposed individuals are combined with those with low exposure, making interpretation of study results difficult.[44,49,111,113-116]

Recognizing the need to study specific chemicals, a few studies have estimated the exposure of individual pesticides with biologically plausible mechanisms that may contribute to the etiology of PD.[59,61,66,91,117] However, humans are rarely exposed to single pesticides mutually exclusive of each other, rather they are more often exposed to multiple environmental chemicals.[53]Thus, it may be difficult to detect the effects of specific chemicals alone and research should aim towards estimating the effect of combinations of pesticides on the risk of PD. A few epidemiological studies that have attempted to address the issue of exposure to multiple pesticides by focusing on chemicals that are structurally related, such as organophosphates.[65-67] Exposure to chemicals that share a common target site have been shown to act in a dose-additive manner, i.e. the mixture of pesticides act on the same target and the final toxicity depends on the relative potency of each pesticide in the mixture.[72]

However, people are more often exposed to pesticides that act on the brain via different mechanisms. This phenomenon has lead researchers to propose the multiple-hit hypothesis, which posits that the brain may be able to compensate for the effects of a single pesticide, but cannot homeostatically regulate itself when exposed to multiple pesticides that act via different mechanisms, sustaining cumulative damage.[118] For example, animal models have documented that organophosphates, organochlorines, paraquat, and dithiocarbamates disrupt the dopaminergic system through a variety of mechanisms, some share the same target site and

others act on different aspects of the dopaminergic system. Inhibition of the electron transport chain within the mitochondria is a common mechanism shared by all of the pesticides mentioned above. Organophosphates have been suggested to possibly inhibit Complex I through IV, paraquat inhibits Complex III, while organochlorines and dithiocarbamates target Complex III. In addition to targeting Complex III, dieldrin, a potent organochlorine, initiates an apoptotic cascade by signaling the mitochondria to release cytochrome c. Another mechanism shared by organophosphates, organochlorines, paraquat, and dithiocarbamates is inducing oxidative stress, which destroys dopaminergic neurons. Organophosphates and organochlorines reduce GSH levels, resulting in an accumulation of lipid peroxides. Paraquat causes an increased production of ROS by either the redox cycling reactions of paraquat with molecular oxygen and/or an NADH-dependent formation of superoxide anions. Dithiocarbamates have been shown to increase the efflux of vesicular DA and disrupt the metabolism of DA, leading to an accumulation of DA's toxic metabolites. Beyond their shared mechanisms, these pesticides also have unique mechanisms that disrupt the dopaminergic system. Organophosphates inhibit AChE, causing dopaminergic neurons to produce ROS as the cell is unable to maintain its energy levels.[36,71] Organophosphates also disrupt microtubule assembly and alter proteins involved in vesicular transport to the axon terminal, causing a build up of vesicles containing DA within the cell. Organochlorines cause the overexpression of DAT and inhibit the uptake of DA into vesicles, leading to an accumulation of DA in the cytoplasm and the oxidation of cytoplasmic DA contributes to oxidative stress. Paraquat up-regulates the accumulation of alpha-synuclein and causes an excitotoxic cascade due to the increased glutamate levels. Lastly, dithiocarbamates disrupt the UPS system, causing an accumulation of damaged proteins associated with PD pathology. Thus PD provides an excellent platform to study the synergistic

effects of multiple pesticides, given the evidence that the biological pathways of these individual pesticides may play a role in the etiology of PD.

In support of the multi-hit hypothesis, Silva et al. showed that there were significant mixture effects of chemicals, even though individual chemicals were present below their NOEC (no observed effect concentration) level.[119] Animal studies confirm the observation that chemicals can act synergistically as mice injected with a combination of paraquat and maneb exhibited Parkinson-like movement deficits.[120] A recent case-control study conducted in the Central Valley of California, also found that those exposed to both maneb and paraquat were at higher risk of developing PD than those exposed to either chemical alone.[22] Additionally, Richardson et al. demonstrated that developmental exposure to dieldrin resulted in the susceptibility of dopaminergic neurons to MPTP.[121]

2.3. Exposure Assessment

Although animal studies provide a wealth of evidence that a number of pesticides may be involved in the etiology of PD, epidemiological studies have not reported conclusive evidence that links exposure to pesticides with an increased risk of PD. The limitations of current pesticide exposure assessment methods play a role in the equivocal nature of the published literature regarding pesticides and PD.

One such limitation is the issue of recall bias. Due to the fact that PD is a rare disease, the case-control study is the most efficient study design to use.[48] Pesticide application records are rare and thus, exposure estimation often relies on self-reported pesticide exposure. Recall bias becomes problematic in case-control studies because subjects are aware of their case status and cases may be more prone to report pesticide exposure, leading to an exaggerated association

between pesticide exposure and PD. Alternatively non-differential exposure misclassification may arise as people often are not able to remember their pesticide exposure history or may not even be aware that they were exposed to pesticides, most likely biasing the association between pesticide exposure and PD towards the null.[48,122]

2.3.1. GIS-based Models

Geographic Information System (GIS) is a set of tools that is used to collect, store, retrieve, transform, and display spatial data extracted from cartographic sources, earthbound survey, and remote sensing.[123,124] Researchers realized that by combining information from multiple data sources such as ground survey data, vectorized maps, and satellite images, they were able to answer questions about the spatial distributions of environmental exposures and their relationship to human health.[123] GIS can be divided up into three main components: data inputs, database transformation, and data output.[123] Data inputs include information from traditional maps and locations geocoded by Global Positioning System (GPS) that make up the GIS database.[123] Database transformation includes the transformation of the coordinate system of input coverages (data layers), data extraction, data overlaying, and data management. Data output involves the creation of new maps synthesized from the manipulation of different layers of maps and data stored GIS database.[123] Like JEMs, GIS-based models have distinct advantages over self-reported data because they rely on pesticide application records rather than subject recall of their pesticide application history. Due to its reliance on recorded data, GIS-based models suffer from less exposure misclassification than JEMs, which rely on experts to assign levels of exposure to subjects based on job information. However, misclassification may be introduced if data inputs are inaccurately geocoded or the maps and records used by GIS are faulty.

An advantage of using GIS is that it allows researchers to estimate environmental exposure in many various ways. Xiang et al. used GIS to assess the relationship between pesticide exposure and low birth weight in Weld County, Colorado.[125] Satellite images were used to identify crop patterns, which were then converted into GIS coverages to be analyzed. Maternal addresses were then geocoded and digitized into another GIS coverage with vital statistical information such as gender, birth weight, gestational age, mother's age, education and smoking during pregnancy. Circular buffers of 300 meters and 500 meters were drawn around each address and the percentage of land covered by crops within each buffer was considered a surrogate for pesticide exposure. Researchers conducted regression analysis and found that low birth weight appeared to be associated with crop patterns within 300 m around mother's residences ($p=0.058$) but not 500 m ($p=0.38$). Regression models employed by researchers do not imply causation or temporal relation,[48] but nevertheless it is worthwhile to note the exposure assessment methodology. A validation study of using satellite imagery to identify crop type and address geocoding was conducted by Ward et al. in Adams, Buffalo, and Hall counties in Nebraska.[126] Pesticide use was mapped in the study area by identifying crop fields using a satellite image and calculating probabilities of pesticide use by crop type and then determining the proportion of land within 500 m buffers of residences. After comparing their crop classification with historical records of land cover from Nebraska Farm Service Agencies, average accuracy of crop classification was 78% with accuracies ranging from 68% for pastures and 90% for corn. Address geocoding was also relatively successful with 85% of addresses successfully geocoded. Cornelis et al. created two exposure indicators by weighting crop area and recorded pesticide use by the distance to a subject's home for 20 years using subject's residential history.[127] The cut-off distance for these indicators was set at 5000 m to account for

the drift and volatilization of applied pesticides. They were not able to detect any significant association between crop and pesticide indicators and bladder cancer. This study only accounted for ambient exposure in subjects' residence and was not able to measure ambient exposure at workplaces due to the inability of subjects to recall their previous occupational addresses, which may have contributed to exposure misclassification. These studies demonstrate that it is feasible to use GIS to estimate ambient exposure to agriculturally applied pesticides with various methodologies and that the precision of exposure assessment is highly dependent on the quality of inputs used in GIS-based models.

Chapter III: PEG and CGEP Study

3.1. PEG and CGEP Study: Study Design

3.1.1. PEG Study: Study Design

The Parkinson's, Environment and Gene (PEG) study is a population-based, case-control study of PD etiology conducted in the Central Valley of California since 2001-2007. Incident cases of Parkinson's disease and population controls were recruited from Fresno, Kern and Tulare counties and were chosen to represent mixed urban/rural locales including agricultural areas of high pesticide use.

PEG specifically aims to (1) identify all recently diagnosed PD patients in three mostly rural and agricultural California counties between January 1, 2001 and January 1, 2007 and confirm PD diagnosis; (2) select local population controls marginally matched to cases in age, gender, ethnicity and county of residency, and unaffected sibling controls; (3) collect and bank

blood samples for DNA extraction and determine polymorphisms for candidate "susceptibility" genes; (4) use geographic information system technology and the California Pesticide Use Report (PUR) database to estimate occupational and residential exposures; and (5) examine metabolic gene polymorphisms and environmental exposure interactions.

3.1.2. CGEP Study: Study Design

The Center for Gene-Environment Studies in Parkinson's disease (CGEP) study was conducted between September 15, 2008 and August 31, 2010. The CGEP study aims to identify novel pathogenic mechanisms of PD based on understanding the cellular pathways disrupted by environmental toxicants, such as pesticides. The study also recruited additional population-based control participants also from Fresno, Kern and Tulare counties in order to increase our statistical power to detect associations between specific pesticides and PD risk. We employed a household cluster sampling strategy selecting 1,388 clusters, each comprised of five addresses, from residential parcel maps acquired from tax assessor's offices in the three counties. Specifically, we selected one index household randomly and four neighboring households to form a cluster. All five residences were approached by trained field workers to determine if any household members met the eligibility criteria for recruitment.

3.2. PEG and CGEP Studies: Study Population

3.2.1. Case Definition

Cases were all individuals who: 1) had been diagnosed with PD for the first time by a physician within the past 3 years; 2) were residents of Fresno, Kern, or Tulare Counties and had lived in California for at least 5 years; 3) had been seen by UCLA movement disorder specialists

and confirmed as having clinically "probable" or "possible" PD; 4) did not have any other diagnosed neurological condition or serious psychiatric condition, such as bipolar disorder, schizophrenia or dementia prior to motor symptom onset; 5) were not in the last stages of a terminal illness; 6) had agreed to participate in the study.

3.2.2. Case Recruitment

The PEG Study recruited PD cases from the populations of Fresno, Tulare, and Kern Counties, California with the help of neurologists practicing in or nearby this region. Altogether, 28 (90%) of the 31 practicing neurologists in these counties who provide care for PD patients participated in this study. Furthermore, we also collaborated with large medical groups (i.e. Kaiser Permanente, Kern and Visalia Medical Centers and the Veteran's Administration), Parkinson's disease support groups, local newspapers and local radio stations that broadcast public service announcements. Participating neurologists notified their PD patients about the study through mailings and/or passing out study brochures to patients at office visits. PD support groups in Bakersfield, Visalia, and Fresno distributed information about our study to their members and our study's neurologists and personnel attended local support group meetings in order to recruit new-onset patients.

Of 1,167 PD patients initially identified through neurologists, large medical groups, and public service announcements, 604 did not meet eligibility criteria: 397 had their initial PD diagnosis more than 3 years prior to recruitment, 134 lived outside the tri-county area at the time of recruitment, 51 had a diagnosis other than PD, and 22 were too ill to participate. Of the 563 eligible cases, 90 could not be examined (56 declined to participate or moved away, 18 had become too ill to be examined, and16 died prior to the scheduled appointment). Of the 473 subjects examined by a University of California at Los Angeles movement disorder specialist, 94

did not meet published criteria for idiopathic PD[128,129] when examined or re-examined during the initial study period, an additional 13 were reclassified as not idiopathic PD during our follow-up study,[130] and 6 subjects withdrew between examination and interview. Of the remaining 360 cases, 3 participants were excluded due to having been first diagnosed with PD after January 2007 for a total of 357 cases.

3.2.3. Population Control Recruitment

Population-based controls were recruited initially from Medicare lists (2001) and, after the Health Insurance Portability and Accountability Act (HIPAA), from residential tax assessor records from the tri-county area. Two sampling strategies were implemented to increase enrollment success and achieve representativeness of the control population: random selection of residential parcels enrolled via mail and phone and clustered random selection of five households enrolled via in-person visits, described in detail elsewhere.[22]

Of the 1,212 potential controls contacted through the first PEG sampling strategy, 457 were ineligible: 409 were <35 years of age, 44 were too ill to participate, and 4 primarily resided outside the study area. From 755 eligible population controls, 409 declined, became too ill to participate, or moved out of the area after screening and prior to enrollment; 346 population controls enrolled from the first sampling strategy. Of the 346 controls 341 provided all information needed in this analysis. Of the 4,756 individuals screened for eligibility through the second sampling strategy, 3,515 were ineligible (88% due to age criteria). From 1,241 eligible population controls, 634 declined participation; 607 population controls enrolled under the second CGEP sampling strategy, but 183 of those controls completed only an abbreviated questionnaire, 2 did not provide ethnicity information, 11 did not complete the residential or occupational histories in the full questionnaire thereby necessitating their exclusion from this

analysis. There were 24 participants recruited under the second sampling strategy that did not provide family history of PD and were assumed to not have a family history of PD. Additionally, for some clusters more than one control was recruited in the second round of control recruitment, potentially introducing biases due to correlated exposures if those controls lived in the same areas during the study period between 1974-1999. However, after selecting one control at random and excluding the rest from each cluster (n=23) the results remained similar suggesting that the historical exposures of controls within the same clusters were not correlated. Therefore all controls recruited under the second sampling strategy were included. In total, 752 controls provided all information necessary for inclusion in this analysis.

3.3. PEG and CGEP Study: Data Collection

UCLA movement disorder specialists examined all potential PD cases, administered the motor portion of the United Parkinson's Disease Rating Scale (UPDRS), and assigned cases a score on the Modified Hoehn and Yahr Staging Scale. Whenever possible, examinations were performed when cases were in the "off" state (i.e. had not taken levodopa or other PD medications) for at least 12 hours. Cases provided blood or saliva samples, completed medical history forms, provided residential and occupational histories, and responded to the 15-Question Yesavage Geriatric Depression Scale (GDS) and the Mini Mental State Exam (MMSE). Residential, occupational history, residential and commercial pesticide use, smoking status, and family history of PD were collected via telephone by trained interviewers. A secondary telephone interview was conducted to follow-up on missing or unclear data.

3.4. PEG and CGEP Study: Demographics

We recruited a total of 1109 subjects into the PEG Study, 357 cases and 752 controls. Table 1 summarizes the demographic variables of interest for our analysis. We enrolled a similar number of males (50.1%) and females (49.9%) into the study. 73.3% of subjects were over the age of 60 at diagnosis for cases and at interview for controls. The average age of cases was 68.3 (range 34-88) and the average age of controls was 66.9 (range 35-99). Cases were more likely to have never smoked (52.4%) than controls (48.1%)

Table 3.1. PEG and CGEP Population Demographics

	Case		Control	
	(N=357)	%	(N=752)	%
Age (Mean and Range)*	68.3 (34-88)		66.9 (35-99)	
<= 60	75	21.0	221	29.4
> 60	282	79.0	531	70.6
Missing				
Gender				
Female	152	42.6	401	53.3
Male	205	57.4	351	46.7
1st Deg. Relative with PD				
No	305	85.4	689	91.6
Yes	52	14.6	63	8.4
Race				
White	287	80.4	526	70.0
Non-White	70	19.6	226	30.0
Education				
12 yrs	96	26.9	156	20.7
<12 yrs	66	18.5	111	14.8
>12 yrs	195	54.6	485	64.5
Smoker Status				
Never smoker	187	52.4	362	48.1
Ex smoker	150	42.0	304	40.4
Current smoker	20	5.6	86	11.5

* Age represents age at PD onset for cases and age at interview for controls

3.5. Exposure Assessment

3.5.1. GIS-based Environmental Pesticide Exposure Assessment

We employed a Pesticide Use Reporting (PUR) and GIS-based model that relies on circular buffers drawn around an address to estimate exposure at work places or residences due to the drift from agriculturally applied pesticides.[131-133] A technical discussion of our GIS-based approach is provided elsewhere,[134] here we briefly summarize the data sources and exposure modeling process.

3.5.2. Pesticides Use Reporting (PUR)

PURs are recorded by the CA DPR for any commercial application of restricted-use pesticides (defined as "agents with harmful environmental or toxicological effects"), and since 1990 for all commercial uses of pesticides regardless of toxicological profile. The location of each PUR record is referenced to the Public Land Survey System (PLSS), a nationwide grid that parcels land into sections at varying resolutions. Each PUR record includes the name of the pesticide's active ingredient, the poundage applied, the crop and acreage of the field, the application method, and the date of application.

3.5.3. Land Use Maps

Because the PUR records only link an agricultural pesticide application to a whole PLSS grid section, we added information from land use maps to more precisely locate the pesticide application as described in detail elsewhere.[135] The California Department of Water Resources (CDWR) periodically (every 7 to 10 years) performs countywide large-scale surveys of land use and crop cover allowing us to identify the location of specific crops within each PLSS grid

section. Digital maps from more recent (1996 to 1999) surveys are available and paper maps were manually digitized for earlier periods (1977 to 1995). The 1977 land use survey was conducted closest in time to 1974 when PUR became available. We constructed historical electronic maps of land use and crop type, and using the PLSS grid section and crop type reported on the PUR, we allocated pesticide applications to an agricultural site to which we assigned a GIS-based location.

3.5.4. Geocoding

We obtained historical occupational and residential addresses from study participants. Addresses located within Fresno, Kern, and Tulare County (tri-county area) during the period of 1974-1999 were automatically geocoded to TigerLine files (Navteq, 2006), and then manually resolved in a multi-step process similar to that described by McElroy.[136] Occupational addresses tend to be recalled with less accuracy (i.e., at the zip code, city, state, or regional centroid level) and thus geocoded less precisely than residential addresses. However, the geocoding quality of addresses for cases and controls were similar with 27% of cases and 23% of controls spending 50% or more of the years between 1974-1999 at very precisely geocoded occupational addresses (i.e., at the level of a parcel unit, street address, or street intersection) while 38% of cases and 48% of controls spent 50% or more of these years at very precisely geocoded residential addresses. This suggests that geocoding precision is not likely to account for difference in the estimated effects.

3.5.5. Deriving Pesticide Exposure Estimates at Occupational and Residential Addresses Using Circular Buffers

Employing our GIS-based system, we combined PUR data, land use maps,[134,135] and geocoded address information to produce estimates of pesticide exposure within a set distance of a subject's work place or residence for the 1974 to 1999 period covered by the PUR data. As suggested by previous literature, exposures at each address and for each pesticide were derived for a fixed buffer with a radius of 500 meters drawn around each address for each year of the 25 year period. This method is generally supported by the finding that measurable concentrations of pesticides can be found within an area for both ground and aerial applications.[125,131,133,136-138] For each pesticide and each address radius, pounds of pesticide applied annually were summed for each buffer and weighted by the proportion of treated acreage in each buffer, resulting in an average amount of specific pesticide applied during 1974-1999. Thus, our GIS-based model estimates ambient exposures to specific types and amounts of agricultural pesticides applied nearby work places or residences, rather than exposures due to active handling and applying of pesticides during farming operations or residential applications as would be obtained in conventional interviews based on subject recall of pesticide use.

We considered people who could not be assigned pesticide exposure due to poor geocoding precision as unexposed. Since cases and controls have similar geocoding precision we do not expect there to be differential misclassification between cases and controls. By taking this conservative approach we considered cases that do not have pesticide exposure information as "not exposed to pesticides" and expect our current results to be attenuated to the null, because we hypothesize that cases will more likely be exposed to pesticides than controls.

Chapter IV: Parkinson's Disease Risk from Ambient Exposure to Maneb, Ziram, and Paraquat

This chapter of the study was conducted and published before the CGEP population controls were added to the study and also included five cases who were later determined to have been misdiagnosed for PD. Thus, the study population in Chapter IV will differ from the study populations of Chapter V and VI.

4.1. Abstract

Due to the heavy and expanding agricultural use of neurotoxic pesticides suspected to affect dopaminergic neurons, it is imperative to closely examine the role of pesticides in the development of Parkinson's disease (PD). We focus our investigation on pesticide use in California's heavily agricultural central valley by utilizing a unique pesticide use reporting system. From 1998 to 2007, we enrolled 362 incident PD cases and 341 controls living in the Central Valley of California. Employing our geographic information system model, we estimated ambient exposures to the pesticides ziram, maneb, and paraquat at work places and residences from 1974-1999. At workplaces, combined exposure to ziram, maneb, and paraquat increased risk of PD three-fold (OR: 3.09; 95%CI: 1.69,5.64) and combined exposure to ziram and paraquat, excluding maneb exposure, was associated with a 80% increase in risk (OR:1.82; 95% CI: 1.03,3.21). Risk estimates for ambient workplace exposure were greater than for exposures at residences and were especially high for younger onset PD patients and when exposed in both locations. Our study is the first to implicate ziram in PD etiology. Combined ambient exposure to ziram and paraquat as well as combined ambient exposure to maneb and paraquat at both workplaces and residences increased PD risk substantially. Those exposed to ziram, maneb, and paraquat together experienced the greatest increase in PD risk. Our results suggest that

pesticides affecting different mechanisms that contribute to dopaminergic neuron death may act together to increase the risk of PD considerably.

4.2. Introduction

Parkinson's disease (PD) is a common movement disorder associated with the degeneration of dopaminergic neurons of the substantia nigra. PD has an estimated annual incidence of approximately 17 per 100,000 and an increasing prevalence worldwide due to the growth of an aging populations.[5] Recently, a number of animal studies have suggested biologic mechanisms for specific pesticides that may increase PD risk. Paraquat has been shown to damage dopaminergic neurons by promoting oxidative stress and cell death.[90,96,100,139] Exposure to manganese ethylene-bis-dithiocarbamate, the major active ingredient in the dithiocarbamate fungicide maneb, selectively produces dopaminergic neurodegeneration in mice by disrupting mitochondrial function, increasing oxidative stress, and inhibiting proteasomal function.[101,105] Ziram, another dithiocarbamate, has been shown to cause dopaminergic neuron damage in cell culture by inhibiting the E1 ligase of the ubiquitin proteasome system (UPS).[106] Recent animal studies reported that the dopaminergic toxicity of paraquat is enhanced when co-administered with maneb.[118,140] These studies suggest that different toxins may potentially act together and contribute to PD pathology via different pathways linked to dopaminergic neurodegeneration.

The impact of pesticide exposures on humans in agricultural communities is of special concern. Not only are pesticide applicators disproportionately exposed to pesticides due to infrequent use of personal protective equipment and improper pesticide mixing and application, but those living and working near farms are also exposed due to drifting pesticide spray[141-143]. Even though the association between PD, farm work, and pesticide exposures is supported by the literature,[56,58,144] very few studies to date have reported findings for specific chemical

agents.[22,55,59,65,91,117,145] Many studies in human populations employed a case-control design that lends itself to recall bias when pesticides are assessed retrospectively via self-report.[55,146] Occupational cohort studies of PD to date have been limited by a paucity of PD cases handling specific pesticides or relying on participant recall to obtain data on specific pesticides.[59]

We accessed data from the Pesticide Use Report (PUR) system maintained by California's Department of Pesticide Regulation (CA DPR) and used a geographic information system (GIS) to assess ambient exposures to specific pesticide.[134] For the first time, we assess ambient exposures to ziram, maneb, and paraquat derived from occupational in addition to residential addresses. We focus on ziram because it is structurally related to maneb and is a more potent inhibitor of the UPS.[106]

4.3. Methods

All procedures described have been approved by the UCLA-IRB for human participants and informed consent was obtained from all participants.

4.3.1. Case and Control Recruitment

We recruited persons with PD and population controls from Fresno, Tulare, and Kern counties ("tri-county" area), largely agricultural areas in Central California, details are provided elsewhere.[147] Briefly, PD cases newly diagnosed between January 1998 and January 2007, residing in the tri-county area and living in California for at least 5 years prior to diagnosis were recruited into our study within 3 years of diagnosis. We collaborated with practicing neurologists, Kaiser Permanente, Kern and Visalia Medical Centers and the Veteran's Administration, Parkinson's disease support groups, local newspapers, and radio stations that broadcast public service announcements to recruit participants in the tri-county area.

Of the 1,167 PD cases we invited and who responded to participate in the study, 604 were not eligible: 397 had been diagnosed more than 3 years prior to contact, 51 denied a PD diagnosis, 134 lived outside the tri-county area, and 22 were too ill to participate. Of the 563 cases found eligible, 473 were examined by a UCLA movement disorder specialist at least once and confirmed as having clinically "probable" or "possible" PD; the remaining 90 potential cases could not be examined or interviewed (54% withdrew, 32% were too ill or died, and 14% moved away). Among those examined, we excluded 83 for whom we were unable to confirm a diagnosis of idiopathic PD, leaving us with 390 cases. We were able to re-examine 71% of the cases and excluded another 21 participants misdiagnosed with PD. Of the remaining 369 cases, 362 provided all information needed for analyses.

Initially controls older than 65 years of age were identified from Medicare enrollee lists in 2001 and were invited to participate in our study, but due to Medicare prohibiting the continued use of enrollees after HIPAA implementation, we changed our recruitment plan and recruited the remaining 70% of our controls from randomly selected residential units (parcels) from tri-county tax assessor records. We mailed letters of invitation to a random selection of parcels and also attempted to identify head-of-household names and telephone numbers for these parcels using marketing companies' services and Internet searches. We contacted 1,212 potential controls by mail and/or phone for eligibility screening to recruit one person per household. Eligibility criteria were: 1) not having PD, 2) being at least 35 years of age, 3) currently residing primarily in one of the three counties, and 4) having lived in California for at least 5 years prior to the screening. Of the 457 ineligible controls, 409 were too young, 44 were terminally ill and 4 primarily resided outside the study area. Of the 755 eligible population controls, 409 declined

participation, were too ill or moved out of the area before honoring an appointment and 346 were enrolled, and 341 provided all information needed for analyses.

For all study participants, we conducted telephone interviews to obtain demographic and exposure information.

4.3.2. GIS-based Ambient Pesticide Exposures Assessment

Employing our GIS-based system, we combined PUR data, land use maps, and geocoded address information[134,135] to produce estimates of pesticide exposure within a 500-meter radius buffer around participants' occupational and residential addresses as suggested in previous literature.[131,133,136] A technical discussion of our GIS-based approach is provided elsewhere, here we briefly summarize the data sources and exposure modeling process.[134] In a previous validation study, our GIS-derived measure for organochlorine exposures identified those with high serum dichlorodiphenyldichloroethylene levels with high specificity (87%).[148]

4.3.3. Pesticides Use Reporting

Since 1974, the CA DPR has recorded agricultural application of restricted-use pesticides (defined as "agents with harmful environmental or toxicological effects"), and for all agriculturally applied pesticides from 1990 onwards. The location of each PUR record is referenced to the Public Land Survey System (PLSS), a nationwide grid that parcels land into sections at varying resolutions. Each PUR record includes the name of the pesticide's active ingredient, the poundage applied, the crop and acreage of the field, the application method, and the date of application.

4.3.4. Land Use Maps

Because the PUR records only link an agricultural pesticide application to a whole PLSS grid section, we added information from land use maps to more precisely locate the pesticide application as described in detail elsewhere.[135] Briefly, the California Department of Water Resources periodically (every 7 to 10 years) performs countywide surveys of location and extent of land use and crop cover. We constructed historical electronic maps of land use and crop type from digital maps from recent surveys[149] (1996 to 1999) and manually digitized earliest available paper maps (1977 to 1995). Using the PLSS grid section and crop type reported on the PUR, we further refined pesticide applications using the more detailed land use geography.

4.3.5. Geocoding

We obtained historical occupational and residential addresses from all study participants. Addresses reported for the period of 1974-1999 in the tri-county area were automatically geocoded to TigerLine files (Navteq, 2006), and then manually resolved in a multi-step process similar to that described by McElroy.[136] We considered geocoded addresses as having high accuracy if we were able to geocode to the actual address, a parcel/lot centroid, street centroid, or street intersection. Inaccurately geocoded addresses were considered to be those geocoded at the zipcode, city, county, state centroids, or did not have enough information to be geocoded.

4.3.6. Pesticide Exposure Estimates at Occupational and Residential Addresses

First we combined the PUR data, land use maps, and geocoded address information and created 500 meter buffers around addresses in our GIS for each year in the 26-year period from 1974 to 1999. Then we calculated annual ambient exposures to the individual pesticides, maneb,

ziram, and paraquat, for each participant by summing the pounds of pesticides applied in each buffer and weighting the total poundage by the proportion of the acreage treated. For each of the three pesticides examined in this study, we summed the annual pounds applied per acre to obtain 26 annual exposure values for each pesticide separately for occupational and residential addresses.

Average pesticide exposures were then calculated for the following exposure time windows: 1974-1999, 2) 1974-1989, 3) 1990-1999 to address a possible extended induction period for PD and assess the influence of age at exposure. A participant was considered exposed to a particular pesticide when the pounds per acre measured was greater than zero during the time window. We created exposure measures for single and combined pesticides by creating categories of co-exposures to different pesticides. Participants that did not work or live in the tri-county area between 1974 and 1999 could not be assigned an exposure estimate and were considered unexposed.

In the same manner, we also created exposure estimates for organophosphates and organochlorines, two pesticide classes that also contribute to neurodegeneration.[73,83] Participants were considered exposed if they had any exposure to at least one organophosphate or organochlorine pesticide.

4.3.7. Statistical Analysis

We conducted analyses of occupational and residential exposures to maneb, ziram, and paraquat individually and in different combinations. We also conducted analyses stratified by exposure time window and by age. We adjusted for age at diagnosis (cases) or age at interview (controls), sex, ethnicity (White vs. non-White), education (< 12 years, 12 years, > 12 years),

having a 1st degree family member with PD (yes, no), and smoking (current, former, never). We also adjusted for organophosphate and organochlorine exposure in some analyses.

We used SAS 9.1 (SAS Institute Inc., Cary, NC, USA) to perform unconditional logistic regression analyses.

4.4. Results

Study participants were predominantly White, over the age of 60, and a minority reported a family history of Parkinson's disease (Table 4.1). Cases were slightly older than controls, more often male, and had completed fewer years of education. They were also more likely to have never smoked cigarettes or to have stopped smoking.

When assessing combinations of exposure to all three pesticides, combined exposure to all three pesticides at both workplaces (OR: 3.09; 95%CI: 1.69, 5.64) and residences (OR: 1.86; 95%CI: 1.09,3.18) was most strongly associated with PD risk, followed by combined exposure to ziram and paraquat only at workplaces (OR: 1.82; 95%CI: 1.03,3.21) (Table 4.2). Adjustment for exposure to organophosphate (OP) and organochlorine (OC) pesticides, shifted risk estimates slightly towards the null value and increased confidence interval sizes (results not shown), but combined exposure to maneb, ziram, and paraquat at workplaces remained strongly associated with PD risk (OP and OC adjusted OR: 2.61; 95%CI: 1.24,5.48). The rarity of exposure to maneb alone and exposure to ziram and maneb without paraquat precludes estimation of effects for these combinations of pesticides. Exposure to paraquat alone was not associated with PD risk at residences but was associated with an increased risk at workplaces.

When considering the main effects of exposure to ziram, maneb, and paraquat, participants exposed to these three pesticides at both residences and work places experienced a greater increase in risk of PD than those exposed at residences or workplaces only (Table 4.3).

Participants exposed to maneb experienced a similar increase in PD risk when exposed at either workplaces or residences only. However, those exposed to ziram at workplaces only experienced higher PD risk than those exposed at residences only. PD risk did not increase for participants exposed to paraquat at workplaces or residences only.

Combined exposure to ziram and paraquat at workplaces was associated with a two-fold increase in PD risk in the overall 1974-1999 time window (Table 4.4). Furthermore, this combination exposure contributed to PD risk at workplaces in both early and late time windows, while only the early time window contributed to PD risk at residences. These patterns were also observed for combined exposure to maneb and paraquat.

Estimated PD risk increase was generally much larger for those diagnosed with PD at a younger age (age <= 60) (Table 4.5). Younger onset patients that were exposed to a combination of ziram and paraquat at workplaces (OR: 5.98; 95% CI: 1.95,18.32) experienced a greater risk of PD than when exposed at residences (OR: 2.78; 95% CI: 1.10,7.07). Similarly, for younger onset patients, exposure to maneb and paraquat alone and in combination was associated with a much larger risk at workplaces than at residences.

4.5 Discussion

The population-based case-control study of PD we conducted in a heavily agricultural region of California shows that combined exposure to ziram and paraquat, apart from maneb exposure, confers an increased risk for developing PD. Our results suggest that exposure to paraquat, maneb and ziram may act together to increase the risk of PD more strongly than exposure to each individual pesticide alone or exposure to any combination of two pesticides. Only the early time window was important for ambient residential exposures to either ziram and paraquat or maneb and paraquat. In contrast, ambient workplace exposure during the early or

late time window to either ziram and paraquat or maneb and paraquat increased PD risk, suggesting that although there may be a long induction period for these combinations of pesticides, potentially more intense occupational exposures later in life may also contribute to risk of developing PD. Finally, younger participants consistently experienced the greatest risks when exposed to a combination of either maneb and paraquat or ziram and paraquat. We not only confirmed our previous results for residential exposures to paraquat and maneb with our new occupational address based exposure measures,[22] but also observed that risk estimates at workplaces were generally larger than at residences and that exposures at both work places and residences together further increase risks.

The vast majority of previous epidemiological studies relied on self-reported pesticide exposures and thus may suffer from biased exposure assessment as study participants may misreport their historical pesticide use.[50,57,66,150,151] The issue of recall bias is especially problematic when attempting to estimate exposures to specific pesticides via self-report. The Agricultural Health Study cohort[59] attempted to estimate effects for several specific pesticides but found no pesticide or functional group to be more than weakly associated with incident PD, possibly due to the small number of cases who reported exposure to specific pesticides. Furthermore, self-reported pesticide exposure cannot account for risk in those not actively applying pesticides who nevertheless are potentially chronically exposed to pesticides from drift and contact with contaminated dust in heavily agricultural areas.[143]

A strength of our study is that our GIS-based pesticide exposure assessment allowed us to derive pesticide exposure information for participants who work or live near agricultural pesticide applications and may be unknowingly exposed due to pesticide drift. Additionally, our GIS-based methods employing the PUR data is an improvement over pesticide exposure

assessment methods based on recall only, since it identifies the exact type, amount, and location of a pesticide active ingredient applied historically, and eliminates differential recall of exposure according to case status. Another strength is that we were able to obtain exposure data from occupational in addition to residential addresses. Since agricultural pesticides are applied during working hours, exposure estimates at workplaces may more accurately reflect true pesticide exposure and risk estimates are expected to be of greater magnitude if participants are present when pesticides are applied to fields. Finally, our population-based study is the only study to date in which movement disorder specialists examined patients multiple times to confirm diagnoses, thus reducing disease misclassification.

Our GIS-based method, which uses a 26-year average pesticide estimate at participants' occupational and residential addresses, cannot be considered a quantitative measure of exposure because the derived poundage of active ingredient per acre applied does not translate easily into a measure of human neurotoxicity across pesticides or pesticide classes. In addition, pesticides vary in toxicity so fewer pounds of a highly toxic pesticide may have the same effect as greater poundage of a less toxic pesticide. Thus, we considered participants exposed if they experienced any exposure and created mutually exclusive pesticide exposure categories to assess multiple pesticides.

Another limitation is that the accuracy of our GIS-based pesticide exposure estimation relies on the quality of self-reported addresses. Occupational addresses were generally geocoded less accurately than residential addresses and addresses with lower geocoding accuracy tended to be assigned less exposure than accurately geocoded addresses (results not shown). Exposure estimates could only be obtained for participants with an occupational address located in the tri-county area between 1974-1999. Of the 703 participants, 26% of cases and 26% of controls

were missing occupational address information, while only 4% of cases and 4% of controls were missing residential address information. Different from our previously published work,[22] we classified participants with missing data as unexposed to maintain statistical power when assessing the risk of pesticide exposures at occupational addresses. This approach would bias effect estimates towards the null as long as the resulting exposure misclassification is non-differential by case status, as suggested by the comparable percentage of missing address information.

Despite these limitations, we believe that our GIS model provides us with an accurate qualitative indicator of ambient pesticide exposure from applications and drift in close proximity to workplaces and residences. It is unlikely that our GIS-based results are affected by selection bias because participants were likely unaware of their historical ambient workplace or residential exposure to specific pesticides associated with PD risk, thus their enrollment would not be associated with pesticide exposure.

Our study confirms observations from cell culture studies conducted by our research group that implicate ziram in the pathology of PD[106] and is the first epidemiologic study that provides strong evidence in a human population that 1) the combination of maneb, ziram, and paraquat confers a greater risk of PD than exposure to these individual chemicals alone, suggesting the pesticides that affect different mechanisms leading to dopaminergic cell death may act together to increase the risk of PD; 2) exposure to ziram and paraquat increases the risk of PD independent of combined exposures to maneb and paraquat; and 3) ambient exposure derived from workplaces is associated with a greater risk for developing PD than ambient exposure at residences and those exposed at both workplaces and residences experience the greatest PD risk.

4.6. Tables

Table 4.1. Demographic Characteristics of the Study Population

	Case		Control			
	(N=362)	%	(N=341)	%	OR	95% CI
Age (Mean and Range)	68.2 (34-88)		67.6 (34-92)			
<= 60	77	21	87	26	1.00	reference
> 60	285	79	254	74	1.27	(0.89,1.80)
Sex						
Female	156	43	165	48	1.00	reference
Male	206	57	176	52	1.24	(0.92,1.67)
1st Deg. Relative with PD						
No	307	85	303	89	1.00	reference
Yes	55	15	37	11	1.47	(0.95,2.30)
Race						
White	291	80	279	82	1.00	reference
Non-White	71	20	62	18	1.10	(0.75,1.60)
Education						
<12 yrs	68	19	38	11	1.19	(0.72,1.98)
12 yrs	96	27	64	19	1.00	reference
>12 yrs	198	55	239	70	0.55	(0.38,0.80)
Smoker Status						
Never smoker	191	53	146	43	1.00	reference
Ex smoker	151	42	161	47	0.72	(0.53,0.98)
Current smoker	20	6	34	10	0.45	(0.25,0.81)

Table 4.2. Effect estimates (ORs and 95% CIs) for ambient pesticide exposures to paraquat, maneb, and ziram in the Central California Valley study population for the 1974-1999 time window of exposure

	Occupational**				Residential ***			
	Case (N=362)	Control (N=341)	Adjusted OR*	95% CI	Case (N=362)	Control (N=341)	Adjusted OR*	95% CI
Not exposed to paraquat, maneb, or ziram	164	191	1.00	reference	122	136	1.00	reference
Exposed to paraquat, not maneb or ziram	81	78	1.26	(0.86,1.86)	109	125	0.91	(0.63,1.31)
Exposed to maneb, not ziram or paraquat	1	3	-[a]	-[a]	2	1	-[a]	-[a]
Exposed to ziram, not maneb or paraquat	6	6	1.37	(0.42,4.49)	4	3	1.48	(0.32,6.85)
Exposed to ziram and maneb, not paraquat	1	0	-[a]	-[a]	1	0	-[a]	-[a]
Exposed to maneb and paraquat, not ziram	26	21	1.41	(0.75,2.68)	34	21	1.59	(0.86,2.95)
Exposed to ziram and paraquat, not maneb	37	24	1.82	(1.03,3.21)	37	27	1.37	(0.78,2.42)
Exposed to maneb, ziram, and paraquat	46	18	3.09	(1.69,5.64)	53	28	1.86	(1.09,3.18)

* Adjusted for age, sex, education, smoking, family history of PD, and race.

**Pesticide exposure derived from self-reported occupational addresses.

***Pesticide exposure derived from self-reported residential addresses.

[a] Not calculated due to insufficient cell counts

Table 4.3. Effect estimates (ORs and 95% CIs) for ambient exposures to ziram, maneb, and paraquat at residences and workplaces for the 1974-1999 time window of exposure

	Case (N=362)	Controls (N=341)	Adjusted OR*	95% CI
Ziram				
Not exposed to ziram	229	253	1.00	ref
Exposed at residences only	43	40	1.13	(0.70,1.82)
Exposed at workplaces only	38	30	1.52	(0.90,2.58)
Exposed at both residences and workplaces	52	18	3.01	(1.69,5.38)
Maneb				
Not exposed to maneb	236	266	1.00	ref
Exposed at residences only	52	33	1.71	(1.06,2.77)
Exposed at workplaces only	36	25	1.77	(1.02,3.09)
Exposed at both residences and workplaces	38	17	2.26	(1.22,4.20)
Paraquat				
Not exposed to paraquat	101	110	1.00	ref
Exposed at residences only	71	90	0.77	(0.50,1.17)
Exposed at workplaces only	28	30	1.07	(0.59,1.96)
Exposed at both residences and workplaces	162	111	1.50	(1.03,2.18)

* Adjusted for age, sex, education, smoking, family history of PD, and race.

Table 4.4. Effect estimates (ORs and 95% CIs) for ambient exposures to maneb, ziram, and paraquat by time window of exposure

Time window of exposure	Occupational**				Residential ***			
	Case (N=362)	Control (N=341)	OR*	95% CI	Case (N=362)	Control (N=341)	OR*	95% CI
Maneb and paraquat exposure								
1974 - 1999 Overall Time Window								
Not exposed to maneb or paraquat	170	197	1.00	reference	126	139	1.00	reference
Exposed to parauqat, not maneb	118	102	1.37	(0.97,1.94)	146	152	0.98	(0.70,1.38)
Exposed to maneb, not paraquat	2	3	0.96	(0.16,5.99)	3	1	3.21	(0.32,32.68)
Exposed to maneb and paraquat	72	39	2.15	(1.36,3.41)	87	49	1.73	(1.11,2.68)
1974 - 1989 Time Window								
Not exposed to maneb or paraquat	180	212	1.00	reference	144	165	1.00	reference
Exposed to maneb or paraquat	124	96	1.43	(0.99,2.07)	145	137	1.15	(0.81,1.63)
Exposed to maneb and paraquat	58	33	1.82	(1.08,3.07)	73	39	2.05	(1.23,3.40)
1990 - 1999 Time Window								
Not exposed to maneb or paraquat	269	279	1.00	reference	227	228	1.00	reference
Exposed to maneb or paraquat	71	52	1.15	(0.74,1.81)	110	95	0.88	(0.61,1.28)
Exposed to maneb and paraquat	22	10	1.69	(0.74,3.84)	25	18	0.91	(0.46,1.82)
Ziram and paraquat exposure								
1974 - 1999 Overall Time Window								
Not exposed to ziram or paraquat	165	194	1.00	reference	124	137	1.00	reference
Exposed to parauqat, not ziram	107	99	1.30	(0.91,1.86)	143	146	0.99	(0.70,1.41)
Exposed to ziram, not paraquat	7	6	1.65	(0.52,5.17)	5	3	1.75	(0.40,7.62)
Exposed to ziram and paraquat	83	42	2.37	(1.52,3.68)	90	55	1.60	(1.05,2.46)
1974 - 1989 Time Window								
Not exposed to ziram or paraquat	175	211	1.00	reference	144	165	1.00	reference
Exposed to ziram or paraquat	121	90	1.55	(1.06,2.26)	154	139	1.13	(0.80,1.61)
Exposed to ziram and paraquat	66	40	1.71	(1.05,2.78)	64	37	1.79	(1.05,3.05)
1990 - 1999 Time Window								
Not exposed to ziram or paraquat	267	277	1.00	reference	218	227	1.00	reference
Exposed to ziram or paraquat	62	53	1.04	(0.66,1.64)	93	81	1.03	(0.70,1.51)
Exposed to ziram and paraquat	33	11	2.16	(1.01,4.63)	51	33	1.06	(0.61,1.84)

* Adjusted for age, sex, education, smoking, family history of PD, and race; exposure time windows are mutually adjusted for each other.

**Pesticide exposure derived from self-reported occupational addresses.

***Pesticide exposure derived from self-reported residential addresses.

Table 4.5. Effect estimates (ORs and 95% CIs) for ambient exposures to maneb, ziram, and paraquat by age at PD diagnosis for the 1974-1999 time window of exposure

	Occupational**				Residential ***			
	Case (N=362)	Control (N=341)	OR*	95% CI	Case (N=362)	Control (N=341)	OR*	95% CI
Maneb and paraquat exposure								
60 years old or younger								
Not exposed to maneb or paraquat	30	56	1.00	reference	20	38	1.00	reference
Exposed to maneb or paraquat	29	28	1.78	(0.87,3.64)	36	42	1.53	(0.73,3.19)
Exposed to maneb and paraquat	18	3	8.75	(2.31,33.19)	21	7	4.82	(1.69,13.76)
Over 60 years old								
Not exposed to maneb or paraquat	140	141	1.00	reference	106	101	1.00	reference
Exposed to maneb or paraquat	91	77	1.22	(0.82,1.83)	113	111	0.89	(0.60,1.32)
Exposed to maneb and paraquat	54	36	1.48	(0.88,2.50)	66	42	1.28	(0.78,2.09)
Ziram and paraquat exposure								
60 years old or younger								
Not exposed to ziram or paraquat	28	53	1.00	reference	21	38	1.00	reference
Exposed to ziram or paraquat	30	29	1.90	(0.91,3.93)	35	37	1.65	(0.79,3.45)
Exposed to ziram and paraquat	19	5	5.98	(1.95,18.32)	21	12	2.78	(1.10,7.07)
Over 60 years old								
Not exposed to ziram or paraquat	137	141	1.00	reference	103	99	1.00	reference
Exposed to ziram or paraquat	84	76	1.17	(0.76,1.72)	113	112	0.88	(0.59,1.30)
Exposed to ziram and paraquat	64	37	1.93	(1.10,3.03)	69	43	1.38	(0.85,2.26)

* Age stratified models adjusted for age, sex, education, smoking, family history of PD, and race.

**Pesticide exposure derived from self-reported occupational addresses.

***Pesticide exposure derived from self-reported residential addresses.

Chapter V: The Influence of Ambient Exposure to Paraquat, Dithiocarbamate, Organochlorine, and Organophosphate Pesticides on Parkinson's Disease Risk

5.1. Abstract

Previous studies examining the influence of pesticide exposures on Parkinson's disease (PD) often relied on self-report, did not assess multiple and specific pesticide exposures over long periods, or had inadequate sample size. To address these limitations, we developed a geographic information system (GIS)-based exposure assessment tool to estimate historical ambient exposure to agricultural pesticides at residential and occupational addresses. We studied 357 incident PD cases and 752 population controls from the Central Valley of California. Employing our GIS model, we assessed ambient exposures at workplaces and residences for paraquat and pesticides belonging to three distinct classes, organophosphate, organochlorine, and dithiocarbamates during a 26-year period, from 1974-1999. We estimated a greater than two-fold increase in risk of developing PD for participants exposed to organophosphates, organochlorines, dithiocarbamates, and paraquat individually after adjusting for covariates. However after adjusting for other pesticides, only ambient exposure to organophosphates remained strongly associated. There is a high degree of co-exposure to multiple pesticides and pesticide classes in our population and combined exposure may affect PD risk more strongly. We found longer duration of exposure and co-exposure to a large number of different pesticides within the same year to be associated with stronger increases in PD risk. Generally, ambient workplace exposures conferred higher risk of PD than ambient residential exposure.

5.2. Introduction

Parkinson's disease (PD) is a neurodegenerative movement disorder associated with aging and the loss of dopaminergic neurons.[3] Since the discovery that MPP+, a toxic metabolite of 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP), causes parkinsonism, much attention has been devoted to exploring the role pesticides play in the etiology of PD, focusing especially of the herbicide paraquat that structurally resembles MPP+.[4] Paraquat is a commonly used herbicide and along with rotenone is the most commonly used pesticides to generate parkinsonian animal models.[152] However, other widely used pesticides including dithiocarbamates,[101,105] organochlorines,[74,153,154] and organophosphates[36,75] are also suspected to be involved in etiologic pathways leading to PD, for example by enhancing oxidative stress, inhibiting the proteasome, or causing mitochondrial dysfunction in neurons.

Humans are rarely exposed to only one chemical agent; rather, exposures to mixtures or multiple types of chemicals are more common.[53,155] The multiple-hit hypothesis in PD postulates that exposures to multiple chemicals that may interfere with different vulnerable cellular components in dopamine neurons (i.e. vesicular transporter, dopamine transporter, proteasome, and microtubule mediated vesicular transport) may act synergistically and cause greater damage by overwhelming the system's ability to homeostatically regulate itself.[156]

Previously, we reported on workplace and residential ambient exposure to ziram, maneb, and paraquat and found that combined exposures to combinations of these pesticides conferred greater risk of PD than exposures to these pesticides alone.[22,157] Here, we will be employing our unique exposure data obtained from a geographic information system (GIS) to addresses whether the risk of PD increases with exposure to paraquat and three pesticide classes, dithiocarbamates,

organophosphates, and organochlorines. We also explore how duration and intensity of pesticide co-exposures affect PD risk.

5.3. Methods

All of our research procedures described were approved by the UCLA IRB for human subjects. Informed consent was obtained from all participants.

5.3.1. Case and Control Recruitment

Our case-control study enrolled participants from the largely rural and agricultural Kern, Tulare, and Fresno counties in central California. Incident idiopathic PD patients were enrolled between January 1, 2001 and January 1, 2007 and population-based controls between January 1, 2002 and December 31, 2010. Subject recruitment methods[22,158] and case definition[147,159] have been described in detail elsewhere.

Of 1,167 PD patients identified through neurologists, large medical groups, and public service announcements, 604 did not satisfy eligibility criteria: 397 had their initial PD diagnosis more than 3 years prior to recruitment, 134 lived outside the tri-county area during study recruitment, 51 had a diagnosis other than PD, and 22 were too ill to participate. There were 563 eligible cases where 90 could not be examined (56 declined to participate or moved away, 18 had become too ill to be examined, and 16 died prior to the scheduled appointment). University of California at Los Angeles movement disorder specialists examined 473 subjects and found that 94 did not meet published criteria for idiopathic PD[128,129] when examined or re-examined during the initial study period, an additional 13 were reclassified as not idiopathic PD during our follow-up study,[130] and 6 subjects withdrew between examination and interview. Of the remaining 360

cases, 3 participants were excluded due to being diagnosed with PD after January 2007 for a total of 357 cases included in this study.

Population-based controls were recruited initially from Medicare lists in 2001. However, after the Health Insurance Portability and Accountability Act (HIPAA) was enacted controls were recruited from residential tax assessor records from the tri-county area. Two sampling strategies were implemented to increase enrollment success and achieve representativeness of the control population: random selection of residential parcels enrolled via mail and phone and clustered random selection of five households enrolled via in-person visits, described in detail elsewhere.[22]

Of the 1,212 potential controls contacted through the first sampling strategy, 457 were ineligible: 409 were <35 years of age, 44 were too ill to participate, and 4 primarily resided outside the study area. From 755 eligible population controls, 409 declined, became too ill to participate, or moved out of the area after screening and prior to enrollment; 346 population controls enrolled from the first sampling strategy. Of the 346 controls 341 provided all information needed in this analysis. Of the 4,756 individuals screened for eligibility through the second sampling strategy, 3,515 were ineligible (88% due to age criteria). From 1,241 eligible population controls, 634 declined participation; 607 population controls enrolled under the second sampling strategy, but 183 of those controls completed only an abbreviated questionnaire and 11 did not complete the residential or occupational histories in the full questionnaire thereby necessitating their exclusion from this analysis. There were 24 participants recruited under the second sampling strategy that did not provide family history of PD and were assumed to not have a family history of PD. Additionally, for some clusters more than one control was recruited in the second round of control recruitment, potentially introducing biases due to correlated exposures if

those controls lived in the same areas during the study period between 1974-1999. However, after selecting one control at random and excluding the rest from each cluster (n=23) the results did not change suggesting that the historical exposures of controls living within the same clusters before the year 2000 did not affect results. Therefore all controls recruited under the second sampling strategy were included. In total, 752 controls provided all information necessary for inclusion in this analysis.

We conducted telephone interviews to obtain demographic, covariates, as well as residential and occupational address information from all study participants.

5.3.2 GIS-based Environmental Pesticide Exposure Assessment

Employing our GIS-based system, we combined PUR data, land use maps, and geocoded address information[134,135] to produce estimates of pesticide exposure within a 500-meter radius circular buffer around participants' occupational and residential addresses as suggested in previous literature.[131,133,136] A technical discussion[134] and a detailed description[158] of our GIS-based approach has been provided elsewhere.

Briefly, since 1974 the CA Department of Pesticide Regulations (CA DPR) has collected pesticide use reports (PURs) for commercial application of restricted-use pesticides (defined as "agents with harmful environmental or toxicological effects"), and starting in 1990 for all pesticides. Each PUR record included the name of the pesticide's active ingredient, the poundage applied, the crop and acreage of the field, the application method, and location and date of application. Our computer model combines data from PURs with California Department of Water Resources (CDWR) land use maps, and historical occupational and residential addresses from all study participants to estimate ambient pesticide exposure. Annual exposure estimates were calculated by summing the pounds of pesticide applied in each 500 meter buffer

surrounding the occupational or residential address and weighting the total poundage by the proportion of acreage treated within the buffer. The annual exposure estimates were then averaged across the 26-year study period from 1974 to 1999. We included a pesticide from the organophosphate (OP), organochlorine (OC), or dithiocarbamate (DTC) class if five or more controls or cases were exposed to any amount of that pesticide for a total of 36 OPs, 9 OCs, 7 DTCs, and paraquat (Table 5.5).

For the present analysis, we considered a participant exposed to a specific pesticide if their 26-year average was equal to or greater than the median observed in controls. We determined ambient exposure to pesticides separately at workplaces and residences. A participant was considered exposed to a pesticide class if they were exposed equal to or above the median in controls to any one pesticide belonging to that class. In order to evaluate exposure duration and co-exposures to several pesticides within each year, we considered participants exposed in a given year if their exposure to a specific pesticide was equal to or greater than the median of the 26-year average for that pesticide in controls. We then relied on these annual exposure measures to define categories of duration and intensity. Duration categories were based on the controls' median number of years exposed to any pesticide (equal to or above the median of controls' 26 year average) at residences, creating the three categories of 0, 1-8, and 9-26 years of exposure. We defined 'high intensity' co-exposure as exposure to more than five pesticides from any pesticide class (equal to or above the median of controls' 26 year average) in any given year, since exposure to five pesticides was the median number of pesticide co-exposures at residences, creating three categories of 0, 1-5 and >5 pesticide co-exposures within the same year. Participants who did not work or live in the tri-county area at any time between 1974 and 1999 could not be assigned an exposure estimate and were considered unexposed.

5.3.3. Statistical Analysis

Logistic regression analyses were conducted to assess the associations between PD and the GIS-based estimates of ambient pesticide exposures at workplaces and residences using the measures of exposure we created. For all analyses, we adjusted for demographic covariates which included age at PD onset (cases) or age at interview (controls), sex, race (White vs. non-White), education (< 12 years, 12 years, > 12 years), having a 1st degree family member with PD (yes, no), and smoking (current, former, never). We also adjusted analysis for all four pesticide class variables (OP, OC, DTC, and the herbicide, paraquat) along with all other covariates (Table 5.2). Odds ratios and 95% confidence intervals were calculated from models as estimates of PD risk.

We used SAS 9.2 (SAS Institute Inc., Cary, NC, USA) for all analyses.

5.4. Results

Study participants were predominantly White, and over the age of 60; a minority reported a family history of Parkinson's disease (Table 5.1). Cases were slightly older than controls, more often male, and had completed fewer years of education. They were also more likely to have never smoked cigarettes or to have stopped smoking. In this study population, 60% of participants were exposed to two or more pesticides classes. Only 14.5% of the participants were solely exposed to one pesticide class in the 26-year study period. Cases (27.5%) were more likely to be exposed to four pesticide classes than controls (21.4%).

Exposure to OPs, OCs, DTCs, and paraquat at either residences or workplaces was associated with increased odds of developing PD, after adjusting for covariates (Table 5.2). Effect estimates for exposure to these pesticide classes at both workplaces and residences together were consistently larger (greater than 2-fold increase in risk) than for exposures solely

in one or the other place (40% to 2-fold increase in risk). However after mutually adjusting for other pesticide classes, only the OP pesticide class remained associated with increased PD risk. Ambient OP exposure at residences only was associated with a 48% increase in the risk of developing PD (95% CI: 0.96, 2.28) and exposures at workplaces alone conferred a 74% risk increase, however the greatest risk increase was a 2-fold increase associated with exposure at both residences and workplaces. We also found that 70.6% of the participants in this study were exposed to OPs.

Increased duration of ambient pesticide exposure was associated with increasing PD risk (Table 5.3). Participants exposed to pesticides for 1-8 years at workplaces only as well as those exposed to pesticides for 9-26 years at workplaces and 1-8 years at residences experienced a 2-fold increase in PD risk. Participants who were exposed to pesticides for 9-26 years at both residences and workplaces experienced the greatest increase in risk (OR: 2.70; 95% CI: 1.74, 4.19). Thus, overall exposures at workplaces appear to contribute to PD risk more strongly than exposures at residences.

Intensity of ambient pesticide exposure also influenced PD risk in an exposure-response manner (Table 5.4). Specifically, participants exposed to pesticides but never to more than five pesticides in the same year experienced a 63% increase in the risk of developing PD. Participants who were exposed to more than five pesticides in the same year at workplaces and five or fewer pesticides at residences experienced a 2-fold increase in the risk of PD. Participants exposed to more than five pesticides at both workplaces and residences experienced a nearly 3-fold increase in risk of developing PD. Furthermore, participants who were exposed to more than five pesticides in a given year were nearly all exposed to more than one class of pesticides during the study period.

5.5. Discussion

The majority of participants in this population-based case-control study conducted in the Central Valley of California were exposed to two or more pesticide classes. Given the high degree of co-exposures to a number of pesticides from the same as well as different classes and the lack of information about chronic neurotoxicity in humans for most, we cannot determine whether certain pesticides act as confounders. Certain pesticides may be perfect indicators for exposure to another pesticide due to agricultural application practices but exhibit independent effects on PD as well. Alternatively, combined exposures to several pesticides may be needed to induce the neurotoxicity that contributes to neurodegeneration in PD. Due to agricultural practices there will rarely be any individuals exposed to only one or the other chemical, which makes it difficult to assess their independent and combined toxicity in human populations. Thus, merely adjusting for pesticide co-exposures may not be appropriate to assess PD risk and assessing combinations or mixtures of pesticide exposure may be most appropriate. Studies of animal models may be necessary to identify neurotoxic mechanisms relevant to PD. Gene-environment studies will also be helpful in identifying genes that modify the action of pesticides and may suggest biologic pathways for neurotoxicity.

This study estimated a 40% to more than 2-fold increase in risk of PD for ambient OP, OC, DTC and paraquat exposure depending on the exposure location. When mutually adjusting for co-exposures to pesticides from other classes, only OP pesticide estimates remained associated with PD risk in an exposure response manner. However, we found that most participants were exposed to OPs and were exposed to more than one pesticide class hence the possibility that OPs might be perfect proxies for other pesticides, however OPs may also have an independent effect on PD risk that should be a focus for future studies. Additionally, our measure

of ambient exposure duration suggests exposure-response trends with increasing years of pesticide exposures. Ambient co-exposure to pesticides within the same year at both residences and workplaces was associated with stronger PD risk than exposure at either location alone.

A strength of this study is our unique GIS-based pesticide exposure assessment that allowed us to estimate specific pesticide exposure at residences and workplaces from drift and/or contact with contaminated dust/soils in this heavily agricultural region[143]. By utilizing historical PUR records, we were able to identify the specific type, amount, and location of an applied pesticide active ingredient, which is a vast improvement over the majority of previous studies[50,57,66,150,151] that relied on less accurate exposure assessment or subject recall only. Our GIS-based pesticide exposure assessment model also allowed us to estimate pesticide exposure by the year the exposure occurred. This allowed us to take into consideration the timing, correlation, and duration of pesticide exposures.

A limitation of our methods is that our pesticide exposure estimates derived from our GIS-based model cannot be considered a quantitative measure of exposure because the derived poundage of active ingredient per acre applied does not translate easily into a measure of human neurotoxicity across pesticides or pesticide classes. Another limitation is that the precision of our GIS-based pesticide exposure estimation relies on the accuracy of self-reported addresses. Participants tend to recall occupational addresses with less accuracy than residential addresses. This leads to less precise geocoding, confirmed in this study by the geocoding of addresses to zip code, city, county, or state centroids more frequently for occupational than residential addresses when a precise and detailed address was lacking. Very precisely geocoded addresses included those that were geocoded to the actual parcel or street centroid. However, the geocoding quality of the addresses for cases and controls were similar among participants where geocoding data

was available; i.e. 24.6% of cases and 21.2% of controls spent 50% or more of the years between 1974-1999 at precisely geocoded occupational addresses while 37.5% of cases and 48.4% of controls spent 50% or more of these years at precisely geocoded residential addresses. This suggests that geocoding precision is not likely to contribute to the difference in the effect estimates of the cases and controls.

Despite these limitations, our GIS model provides a valid qualitative indicator of pesticide exposure from applications and drift in close proximity to workplaces and residences. It is unlikely that our GIS-based results are affected by selection bias because our participants were not asked to self-report exposures and were most likely unaware of their historical exposures to specific pesticides applied within proximity to their residences and/or work places. Furthermore, there is no reason to suspect that cases and controls would choose to differentially participate in our study based on whether or not they lived and/or worked near agricultural plots for over a quarter of a century.

Animal and cell models have demonstrated that pesticides exhibit specific mechanisms that may contribute to the etiology of PD. It has also been shown that organophosphates inhibit acetylcholinesterase and may increase the acetylcholine level in the basal ganglia.[62] Besides inhibiting acetylcholinesterase, organophosphates also disturb redox processes that inhibit antioxidant enzymes, thereby enhancing lipid peroxidation and oxidative stress,[36] two mechanisms thought to contribute to PD pathogenesis. Organochlorines have long been implicated in the etiology of PD, in part because of post-mortem studies that found brains of PD patients to contain detectable traces of organochlorines more often than those of people who had died of other illnesses.[78,79,160] Dieldrin, a potent organochlorine, has been shown to contribute to apoptotic cell death in dopaminergic neurons via the production of reactive oxygen species,

mitochondrial damage, and release of pro-apoptotic molecules.[74] Exposure to the dithiocarbamate maneb alone has been shown to inhibit proteasomal[105] and mitochondrial function,[101] inducing oxidative stress and damaging cells. Other dithiocarbamates caused dopamine cell injury in cell culture and in vivo by inhibiting the E1 ligase of the ubiquitin proteasome system.[106] Multiple doses of paraquat delivered via subcutaneous injections have been shown to damage dopaminergic neurons[96,100] and make them vulnerable to oxidative stress and cell death.[96,139] Additionally, several animal studies support the multiple-hit hypothesis in PD. A rodent study reported that paraquat co-administered with maneb leads to enhanced dopaminergic neuron toxicity and Parkinson-like movement deficits.[118,120,140] Combined exposure to paraquat and maneb also increases substantia nigra pars compacta neuronal pathology.[161] These studies provide some evidence that co-exposure to several toxins may synergistically contribute to PD pathology, which is consistent with a multiple-hit hypothesis. Our findings that co-exposure to a greater number of pesticides within the same year is associated with increased PD risk further support this hypothesis.

In conclusion, this study adds evidence that increased duration and intensity of ambient pesticide co-exposure increases PD risk in an exposure-response fashion in a human population. Since residents of this heavily agricultural environment in Central California are ambiently exposed to multiple classes of pesticides at residences and workplaces it is important to take the effect of pesticide co-exposures into consideration in future studies.

5.6. Tables

Table 5.1. Demographic Characteristics of the Study Population

	Case		Control	
	(N=357)	%	(N=752)	%
Age (Mean and Range)*	68.3 (34-88)		66.9 (35-99)	
<= 60	75	21.0	221	29.4
> 60	282	79.0	531	70.6
missing				
Sex				
Female	152	42.6	401	53.3
Male	205	57.4	351	46.7
1st Deg. Relative with PD				
No	305	85.4	689	91.6
Yes	52	14.6	63	8.4
Race				
White	287	80.4	526	70.0
Non-White	70	19.6	226	30.0
Education				
12 yrs	96	26.9	156	20.7
<12 yrs	66	18.5	111	14.8
>12 yrs	195	54.6	485	64.5
Smoker Status				
Never smoker	187	52.4	362	48.1
Ex smoker	150	42.0	304	40.4
Current smoker	20	5.6	86	11.4
Pesticide co-exposure				
Not exposed to OP, OC, DTC, or the herbicide paraquat	65	18.2	220	29.3
Exposed to 1 pesticide class	54	15.1	107	14.2
Exposed to 2 pesticide classes	63	17.6	104	13.8
Exposed to 3 pesticide classes	77	21.6	160	21.3
Exposed to 4 pesticide classes	98	27.5	161	21.4

* Age represents age at PD onset for cases and age at interview for controls

Table 5.2. Effect estimates (ORs and 95% CIs) for ambient exposures to organophosphates, organochlorines, dithiocarbamates, and paraquat at residences and workplaces during 1974-1999

Exposure to pesticide class	case	control	Crude OR	OR adjusted for covariates*	95% CI	OR adjusted for pesticides and covariates*	95% CI
Organophosphates							
Not exposed to organophosphates	78	248	1.00	1.00	reference	1.00	reference
Exposed at residences only	75	175	1.36	1.34	(0.91,1.96)	1.48	(0.96,2.28)
Exposed at workplaces only	39	75	1.65	1.69	(1.05,2.74)	1.74	(1.01,3.00)
Exposed at residences and workplaces	165	254	2.07	2.10	(1.51,2.94)	2.11	(1.31,3.41)
Organochlorines							
Not exposed to organochlorines	140	370	1.00	1.00	reference	1.00	reference
Exposed at residences only	60	126	1.26	1.34	(0.91,1.95)	1.26	(0.79,2.02)
Exposed at workplaces only	63	113	1.47	1.40	(0.96,2.05)	1.94	(0.67,1.62)
Exposed at residences and workplaces	94	143	1.74	1.66	(1.18,2.33)	1.03	(0.63,1.70)
Dithiocarbamates							
Not exposed to dithiocarbamates	217	500	1.00	1.00	reference	1.00	reference
Exposed at residences only	47	118	0.92	0.86	(0.58,1.26)	0.71	(0.46,1.09)
Exposed at workplaces only	42	72	1.34	1.41	(0.92,2.17)	1.07	(0.66,1.73)
Exposed at residences and workplaces	51	62	1.90	1.94	(1.27,2.97)	1.41	(0.84,2.34)
Paraquat							
Not exposed to paraquat	190	451	1.00	1.00	reference	1.00	reference
Exposed at residences only	52	122	1.01	1.01	(0.69,1.48)	0.78	(0.50,1.22)
Exposed at workplaces only	50	83	1.43	1.42	(0.94,2.13)	0.89	(0.55,1.45)
Exposed at residences and workplaces	65	96	1.61	1.64	(1.12,2.39)	0.89	(0.52,1.53)

* Adjusted for sex, education, smoking, age, family history of PD, and race

** Adjusted for sex, education, smoking, age, family history of PD, race, and mutually adjusted for each pesticide class

Table 5.3. Effect estimates for the years exposed to ambient exposures to pesticides at residences and workplaces during 1974-1999

	case	control	Crude OR	Adj OR	95% CI
Not exposed to pesticide	46	165	1.00	1.00	reference
Exposed to pesticides for 1-8 years at residences only	41	87	1.69	1.59	(0.96,2.65)
Exposed to pesticides for 9-26 years at residences only	18	34	1.90	1.72	(0.87,3.41)
Exposed to pesticides for 1-8 years at workplaces only	15	26	2.07	2.01	(0.94,4.28)
Exposed to pesticides for 9-26 years at workplaces only	2	6	-	-	-
Exposed to pesticides for 1-8 years at residences and workplaces	70	162	1.55	1.55	(0.99,2.41)
Exposed to pesticides for 9-26 years at residences and 1-8 years at workplaces	35	95	1.32	1.23	(0.73,2.07)
Exposed to pesticides for 1-8 years at residences and 9-26 years at workplaces	33	50	2.37	2.20	(1.25,3.86)
Exposed to pesticides for 9-26 years at residences and 9-26 years at workplaces	97	127	2.74	2.70	(1.74,4.19)

* Adjusted for sex, education, smoking, age, family history of PD, and race

Table 5.4. Effect estimates for ambient exposures to more than 5 pesticides within the same year at residences and workplaces during 1974-1999

Exposure to more than 5 pesticides per year	case	control	Crude OR	Adj OR	95% CI
Not exposed to pesticide	46	165	1.00	1.00	reference
Exposed to fewer than 5 pesticides per year	150	310	1.74	1.63	(1.10,2.41)
Exposed to more than 5 pesticides per year at residences only	46	116	1.42	1.39	(0.85,2.27)
Exposed to more than 5 pesticides per year at workplaces only	43	72	2.14	2.11	(1.26,3.53)
Exposed to more than 5 pesticides per year at residences and workplaces	72	89	2.90	2.90	(1.81,4.67)

* Adjusted for sex, education, smoking, age, family history of PD, and race

Table 5.5. List of Pesticides Included in this Study

Pesticide class	Individual pesticides	Chemical code	Exposed Cases	Exposed Controls
Organophosphates				
	profenofos	2042	46	54
	fenamiphos	1857	43	58
	dialifor	1799	24	21
	methamidophos	1697	56	82
	methidathion	1689	102	174
	acephate	1685	114	147
	leptophos	1676	11	13
	ethephon	1626	100	139
	TEPP	577	3	4
	demeton	566	40	50
	sulfotep	558	5	17
	phosphamidon	482	10	11
	mevinphos	480	76	100
	phosalone	479	32	40
	phorate	478	83	112
	parathion	459	119	205
	naled	418	118	170
	parathion-methyl	394	50	69
	oxydemeton-methyl	382	78	115
	malathion	367	121	155
	phosmet	335	113	195
	azinphos-methyl	314	89	152
	merphos	293	51	55
	ethion	268	47	59
	chlorpyrifos	253	116	209
	disulfoton	230	64	86
	dimethoate	216	167	247
	diazinon	198	142	244
	dioxathion	192	21	26
	tribufos	190	74	120
	DDVP	187	5	5
	carbophenothion	110	23	28
	trichlorfon	88	40	54
	dicrotophos	72	8	8
	bensulide	70	13	13
	monocrotophos	52	77	91
Organochlorines				
	chlorothalonil	677	99	153
	camphechlor toxaphene	594	7	7
	dienochlor	468	5	5
	methoxychlor	384	42	67
	lindane	359	2	9
	dicofol	346	117	206

	dieldrin	259	123	210
	endosulfan	210	22	23
	chlordane	130	68	86
Dithiocarbamates				
	ziram	629	75	124
	zineb	627	16	33
	metam-sodium	616	43	57
	thiram	589	2	5
	metiram	493	4	3
	maneb	369	70	117
	mancozeb	211	64	93
Herbicides				
	paraquat	1601	167	301

Chapter VI: The Association Between Ambient Exposure to Organophosphates and Parkinson's Disease Risk

6.1 Abstract

There is a paucity of studies that have examined associations between specific pesticides and the risk of developing Parkinson's disease. Organophosphates are one of the most commonly used pesticides in the world. They have been shown to promote neurotoxicity by inducing oxidative stress and potentially by disrupting mitochondrial functions, mechanisms implicated in the etiology of PD. This study uses a geographic information system (GIS)-based exposure assessment tool to estimate ambient exposure to 36 commonly used organophosphates from 1974-1999. The study included 357 incident PD cases and 752 population controls living in the Central Valley of California. Ambient exposure to each organophosphate separately increased the risk of developing PD. However, it is difficult to estimate the risk associated with an individual pesticide due to the likelihood that participants were exposed to combinations of these pesticides rather than any one single pesticide. Organophosphates grouped according to different presumed functional toxicities all exhibited similarly elevated risks but exposure-response patterns were observed with an increasing number of these chemicals.

6.2 Introduction

Parkinson's disease is an idiopathic neurodegenerative disease thought to be associated with aging, environmental and genetic factors, and gender.[4,19] Although many studies have found associations between pesticides and PD, much heterogeneity between study findings remains to be explained.[162] While a number of methodological limitations may contribute to conflicting reports in the literature, including difficulties in correctly ascertaining PD case status,

inappropriate control selection, and lack of statistical power, the major limitations of most studies are due to inadequate lifetime exposure assessment for pesticides. Previous studies generally assign pesticide exposure based on self-report, which is likely affected by recall bias. In fact many studies simply revert to defining occupational pesticide exposure as exposure to any type of pesticide or pesticides belong to broad pesticide classes (i.e. insecticides, fungicides, herbicides), further contributing to conflicting findings if some but not all pesticides contribute to PD etiology.[162]

Organophosphate (OP) pesticides represent the largest group of insecticides sold worldwide and are responsible for millions of intentional and unintentional poisonings and thousands of deaths in developing nations.[62,163,164] Some humans who experienced acute OP poisonings also developed signs of parkinsonism, suggesting that OPs may have an effect on the striatal dopaminergic system.[64,165] While the main mechanism of OP toxicity is cholinesterase inhibition and oxidative stress there is some laboratory evidence that OPs may disrupt mitochondrial functions.[164] Since mitochondrial inhibition is a prominent pathological pathway for PD etiology, it is very timely to assess their potential long-term neurodegenerative effects in human populations.

This study aims to assess if specific organophosphates affect PD risk and whether organophosphates found to have certain potentially neurotoxic mechanisms, such as possible disruption of mitochondrial function, contribute most strongly to PD risk.

6.3 Methods

All of the research procedures described in this study were approved by the UCLA-IRB for human subjects. Informed consent was obtained from all participants.

6.3.1. Case and Control Recruitment

This case-control study enrolled incident idiopathic PD patients between January 1, 2001 and January 1, 2007 and population-based controls from the mostly rural agricultural tri-county area (Kern, Tulare, Fresno) in central California between January 1, 2002 and December 31, 2010. Subject recruitment methods[22,158] and case definition criteria[147,159] have been described in detail elsewhere.

Of 1,167 PD patients initially identified through neurologists, large medical groups, and public service announcements, 604 did not meet eligibility criteria: 397 had their initial PD diagnosis more than 3 years prior to recruitment, 134 lived outside the tri-county area at the time of recruitment, 51 had a diagnosis other than PD, and 22 were too ill to participate. Of the 563 eligible cases, 90 could not be examined (56 declined to participate or moved away, 18 had become too ill to be examined, and16 died prior to the scheduled appointment). Of the 473 subjects examined by a University of California at Los Angeles movement disorder specialist, 94 did not meet published criteria for idiopathic PD[128,129] when examined or re-examined during the initial study period, an additional 13 were reclassified as not idiopathic PD during our follow-up study,[130] and 6 subjects withdrew between examination and interview. Of the remaining 360 cases, 3 participants were excluded due to having been first diagnosed with PD after January 2007 for a total of 357 cases.

Population-based controls were recruited initially from Medicare lists (2001) and, after the Health Insurance Portability and Accountability Act (HIPAA), from residential tax assessor records from the tri-county area. Two sampling strategies were implemented to increase enrollment success and achieve representativeness of the control population: random selection of

residential parcels enrolled via mail and phone and clustered random selection of five households enrolled via in-person visits, described in detail elsewhere.[22]

Of the 1,212 potential controls contacted through the first sampling strategy, 457 were ineligible: 409 were <35 years of age, 44 were too ill to participate, and 4 primarily resided outside the study area. From 755 eligible population controls, 409 declined, became too ill to participate, or moved out of the area after screening and prior to enrollment; 346 population controls enrolled from the first sampling strategy. Of the 346 controls 341 provided all information needed in this analysis. Of the 4,756 individuals screened for eligibility through the second sampling strategy, 3,515 were ineligible (88% due to age criteria). From 1,241 eligible population controls, 634 declined participation; 607 population controls enrolled under the second sampling strategy, but 183 of those controls completed only an abbreviated questionnaire and 11 did not complete the residential or occupational histories in the full questionnaire thereby necessitating their exclusion from this analysis. There were 24 participants recruited under the second sampling strategy that did not provide family history of PD and were assumed to not have a family history of PD. Additionally, for some clusters more than one control was recruited in the second round of control recruitment, potentially introducing biases due to correlated exposures if those controls lived in the same areas during the study period between 1974-1999. However, after selecting one control at random and excluding the rest from each cluster (n=23) the results remained similar suggesting that the historical exposures of controls within the same clusters were not correlated. Therefore all controls recruited under the second sampling strategy were included. In total, 752 controls provided all information necessary for inclusion in this analysis.

For all study participants, we conducted telephone interviews to obtain demographic, covariates, as well as residential and occupational address information.

6.3.2. GIS-based Environmental Pesticide Exposure Assessment

Using our GIS-based system, we combined PUR data, land use maps, and geocoded address information[134,135] to produce estimates of pesticide exposure within a 500-meter radius circular buffer around the occupational and residential addresses reported by participants as suggested in previous literature.[131,133,136] A technical discussion[134] and a detailed description[158] of our approach has been provided elsewhere.

Briefly, since 1974 the CA Department of Pesticide Regulations (CA DPR) has collected pesticide use reports (PURs) for agricultural application of restricted-use pesticides (defined as "agents with harmful environmental or toxicological effects"), and starting in 1990 for all pesticides. Each PUR record includes the name of the pesticide's active ingredient, the poundage of pesticide applied, the crop and acreage of the field, the application method, and location and date of application. Our GIS-based computer model combines data from PURs with California Department of Water Resources (CDWR) land use maps, and historical occupational and residential addresses from study participants to estimate ambient pesticide exposure. Annual exposure estimates were calculated by adding the poundage of pesticide applied in each 500 meter buffer surrounding the occupational or residential address and weighting the total poundage by the proportion of acreage treated within the buffer. These annual exposure estimates were then averaged across the 26-year study period from 1974 to 1999. We chose to include a pesticide from the organophosphate (OP) class if five or more cases or controls were exposed to any amount of that pesticide and included a total of 36 OPs in this study (Table 6.5).

For the present analysis, we considered a participant exposed to a specific OP pesticide if their 26-year average was equal to or greater than the median observed in all controls. We determined ambient exposure to individual pesticides separately at workplaces and residences.

Study participants who did not work or live in the tri-county area between 1974 and 1999 could not be assigned an exposure estimate and were considered unexposed.

6.3.3. Statistical Analysis

All selected OPs were analyzed individually and also in groups according to their ascribed mechanisms of toxicity, such as being a carcinogen (carcinogenic: known or possible carcinogen; non-carcinogenic: not likely or not listed as carcinogen), teratogen, endocrine disruptor, and their acute toxicity (toxic: extreme or high toxicity; non-toxic: moderate or slight toxicity) based on the Pesticide Action Network Pesticide Database.[166] We also identified OPs with some evidence of mitochondrial disrupting mechanisms based on the available literature. However, these classifications by mechanism of toxicity were not mutually exclusive i.e. a pesticide may have been assigned multiple mechanisms of toxicity. Logistic regression analyses were conducted to assess associations between PD and the GIS-based estimates of individual and grouped ambient OP exposures at workplaces and residences. We adjusted for age at diagnosis (cases) or age at interview (controls), gender, race (White vs. non-White), education (< 12 years, 12 years, > 12 years), having a 1st degree family member with PD (yes, no), smoking (current, former, never), and included an indicator variable for participants exposed to other pesticides including organochlorines, dithiocarbamates, and paraquat but not OPs.

We used SAS 9.2 (SAS Institute Inc., Cary, NC, USA) to perform unconditional logistic regression analyses.

6.4. Results

Study participants were predominantly White, over the age of 60, and a minority reported a family history of Parkinson's disease (Table 6.1). Cases were slightly older than controls, more

often male, and had completed fewer years of education. They were also more likely to have never smoked cigarettes or to have stopped smoking.

Effect estimates for each individual OP separately are shown in Table 6.2. All OPs investigated were associated with 2-fold or greater risk of developing PD. Generally, exposures at workplaces only or at both residences and workplaces conferred a greater risk than exposures at residences only. Acephate, ethephon, phorate, naled, malathion, merphos, chlorpyrifos, disulfoton, dimethoate, and monocrotophos were the only OPs that were strongly associated with increased risks of developing PD at residences only, workplaces only, and residences and workplaces together.

All groups of OPs classified by mechanism of toxicity appeared to be associated with increased odds of developing PD i.e. none of the groupings seemed more important than others (Table 6.3). Again, exposures at workplaces only or at both residences and workplaces were associated with a greater increase in risk than exposures at residences only. This pattern was also seen for OPs with some suggestion of having mitochondrial disrupting function (Table 6.4). Participants exposed to OPs with some evidence of mitochondrial disrupting properties only at residences exhibited a modest increase in the risk of PD compared to those exposed at workplaces only and those exposed at both residences and workplaces. Moreover, our data suggested a trend where an increasing number of potential mitochondria disrupting OPs at workplaces (p-trend= 0.0014), or at both residences and workplaces (p-trend < 0.0001), were associated with an increasing risk of PD. Similar patters were also observed for OPs that have not been shown to disrupt the mitochondria.

6.5. Discussion

This population-based case-control study conducted in the Central Valley of California found all OPs included in this study to be associated with an increased odds of developing PD. This is the first study to show in a human population that exposures to an increasing number of OPs contributes to a greater risk of developing PD whether or not they were grouped according to presumed functions of toxicity including mitochondria disruption. Thus those classified as teratogens, endocrine disruptors, and carcinogens exhibited similar effects as those assigned mitochondrial disrupting function suggesting that the true mechanisms of neurotoxic action might not be known or that many OPs contributing to these groups have multiple mechanisms of toxicity. Also, we may not be able to distinguish between mechanisms due to OPs exhibiting multiple mechanisms, e.g. seven of the twelve OP endocrine disruptors were also mitochondrial disruptors. Generally, ambient exposure at residences resulted in weaker associations with PD than workplaces or exposure in both locations, suggesting that workplace related ambient exposures and exposure at multiple locations might be higher and act additively to increase risk. This may be due to the fact that most people do not work at their residence and thus are not present when the OPs are applied agriculturally during daytime work hours. The hydrolytic mechanisms of OPs can causes the active ingredients to break down quickly so being present during or shortly after applications of OPs may contribute to a greater increase in the risk of developing PD.[167,168]

A strength of this study is the unique GIS-based pesticide exposure assessment method that was utilized to assess ambient pesticide exposure at residences and workplaces from drift and/or contact with dust and soils contaminated with pesticides in this heavily agricultural region.[143] By utilizing historical PUR records, the specific type, amount, and location of an

applied pesticide active ingredient were identified, a vast improvement over the majority of previous studies that rely on less accurate exposure assessment, such as self-reported exposures. A recent meta-analysis of 46 studies showed a moderate association between general pesticide exposure and PD (sRR: 1.62; 95%CI: 1.40,1.88), however it also found substantial heterogeneity of results between studies included in the analysis.[162] The authors attributed this heterogeneity to unreliable self-reported exposure which may have introduced non-differential misclassification and biased the results towards the null, preventing the detection of existing associations between pesticides and PD risk. Another issue with most of the studies included in the meta-analysis is that the majority of the studies defined pesticide exposure as ever/never exposure to any pesticide or to broad subgroups such as herbicides, insecticides, and fungicides. This approach assumes that all pesticides or pesticides belonging to broad pesticide subgroups similarly affect the risk of developing PD which is unlikely and may introduce bias due to exposure misclassification. Thus, estimates of risk of developing PD based on specific pesticides are probably needed to understand this heterogeneity at least in part.

A limitation of our exposure assessment method is that the precision of the GIS-based pesticide exposure estimation relies on how accurately participants reported their occupational and residential addresses. Occupational addresses tend to be recalled with less accuracy than residential addresses that leads to less precise geocoding. Indeed, occupational addresses were geocoded less precisely (i.e. if a detailed address was lacking, we relied on zipcode, city, county, or state centroids) than residential addresses in this study. However, the geocoding quality of addresses for cases and controls were similar. We found that when assessing geocoded data in our study, 24.6% of cases and 21.2% of controls spent 50% or more of the years between 1974-1999 at very precisely geocoded occupational addresses while 37.5% of cases and 48.4% of

controls spent 50% or more of these years at precisely geocoded residential addresses. This suggests that geocoding precision is not likely to account for difference in the estimated effects. Another limitation is that mitochondrial disrupting properties of OPs have not been widely studied and the majority of the studies that showed mitochondrial disrupting properties in OPs used large experimental doses, which are not reflective of real-world ambient exposures. Thus, the current literature is not sufficient to establish whether individual OPs are mitochondrial disruptors at ambient exposure levels.

Despite limitations, our GIS model provides a valid and high quality indicator of passive pesticide exposure from applications and drift in close proximity to workplaces and residences. It is unlikely that the GIS-based results are affected by selection bias because our participants did not self-report pesticide exposures and were most likely not aware of their historical exposures to specific pesticides applied within 500 meters of their residences and/or workplaces. There is also no reason to suspect that cases and controls would choose to differentially participate in this study based on whether or not they lived and/or worked near agricultural plots during the 26-year exposure period we investigated.

The primary mechanism of OPs is the inhibition of acetylcholinesterase (AChE) leading to an accumulation of acetylcholine at cholinergic synapses and over-stimulation of muscarinic and nicotinic receptors.[62] Although there is evidence that inhibition of AChE may be associated with increased PD risk through cholinergic system overstimulation and consequently cell death,[36,169] OPs are known for a number of non-cholinergic mechanisms of toxicity such as oxidative stress and the inhibition of mitochondrial processes.[164]

Oxidative stress occurs when the body's antioxidant defenses are not able to neutralize an excess of reactive oxygen species (ROS). The brain utilizes a large amount of oxygen that may

lead to an increased production of ROS and is also particularly susceptible to oxidative damage as it is composed largely of polyunsaturated fatty acids.[36] When AChE is inhibited an excessive ROS may be generated when cells are not able to maintain energy levels due to high energy consumption and inhibition of oxidative phosphorylation.[71] Oxidative stress may also originate from the mitochondria when their function is disrupted.[170,171] Dopaminergic neurons in the substantia nigra are more susceptible to insults against the mitochondria, although the mechanism behind this phenomenon is not well understood.[172] It is thought that since dopaminergic neurons are autonomously active, unlike other neurons, there is a greater reliance on oxidative phosphorylation.[172] Thus, it is likely that the inhibition of mitochondrial processes would have a greater impact on dopaminergic neurons.

Besides governing aerobic respiration, mitochondria also are involved in the apoptotic neurodegenerative processes.[164] Apoptosis may be caused by increased production of ROS and the translocation and inhibition of proteins involved in respiration such as cytochrome C.[173] Mevinphos has been shown to disrupt oxidative phosphorylation by causing the dysfunction of Complexes I through IV, which leads to cell death due to ATP depletion.[73] Monocrotophos have also been shown to induce apoptosis in neurons and inhibit metabolism.[173] Chlorpyrifos and chlorpyrifos-oxon exposure resulted in increased mitochondrial length, decreased number of mitochondria, and decreased mitochondria movement in axon at concentrations that did not inhibit AChE.[174] Experiments involving parathion-methyl, malathion, dimethoate, parathion, and dichlorvos showed some mitochondrial disruption, however large experimental doses of these pesticides were used that might have been toxic to all organelles rather than impacting the mitochondria specifically and may not reflect low-dose, chronic, ambient OP pesticide exposure toxicity.[175-180] Taken together, the evidence that OPs cause cell death specifically mediated

through the disruption of mitochondrial functioning is not adequate and our results suggest that while a large number of OPs increased PD risk the mechanisms of action remain to be explored.

In conclusion this study adds strong evidence that OPs are implicated in the etiology of idiopathic PD. Additionally, ambient exposure to OPs at workplaces and combined ambient exposure at residences and work places seem to be especially important. This is the first study to show in a human population that exposure to increasing numbers of OPs is associated with elevated risks of PD. Future studies should further examine possible neurotoxic mechanisms of OPs at low doses that are reflective of real-world ambient exposure.

6.6. Tables

Table 6.1. Demographic Characteristics of the Study Population

	Case		Control	
	(N=357)	%	(N=752)	%
Age (Mean and Range)*	68.3 (34-88)		66.9 (35-99)	
<= 60	75	21.0	221	29.4
> 60	282	79.0	531	70.6
missing				
Gender				
Female	152	42.6	401	53.3
Male	205	57.4	351	46.7
1st Deg. Relative with PD				
No	305	85.4	689	91.6
Yes	52	14.6	63	8.4
Race				
White	287	80.4	526	70.0
Non-White	70	19.6	226	30.0
Education				
12 yrs	96	26.9	156	20.7
<12 yrs	66	18.5	111	14.8
>12 yrs	195	54.6	485	64.5
Smoker Status				
Never smoker	187	52.4	362	48.1
Ex smoker	150	42.0	304	40.4
Current smoker	20	5.6	86	11.5

* Age represents age at PD onset for cases and age at interview for controls

Table 6.2. Effect estimates (ORs and 95% CIs) for ambient exposures to organophosphates at residences and workplaces during 1974-1999

	Residences only				Workplaces only				Residences and Workplaces			
Exposure to pesticide class	case	control	Adjusted OR*	95% CI	case	control	Adjusted OR*	95% CI	case	control	Adjusted OR*	95% CI
Not exposed to pesticides	65	220	1.00	reference	65	220	1.00	reference	65	220	1.00	reference
Profenofos 2042	17	27	2.24	(1.12,4.47)	22	16	4.26	(2.05,8.84)	7	11	1.73	(0.61,4.89)
Fenamiphos 1857	15	29	1.88	(0.91,3.86)	19	19	3.22	(1.56,6.62)	9	10	2.71	(1.04,7.09)
Methamidophos 1697	18	42	1.52	(0.80,2.87)	18	28	2.45	(1.23,4.89)	20	12	4.72	(2.09,10.62)
Methidathion 1689	29	60	1.62	(0.94,2.78)	32	48	2.46	(1.42,4.27)	41	66	2.11	(1.28,3.49)
Acephate 1685	45	64	2.50	(1.52,4.10)	43	45	3.22	(1.91,5.44)	26	38	2.44	(1.34,4.43)
Ethephon 1626	43	68	2.09	(1.28,3.42)	31	38	3.00	(1.68,5.38)	26	33	2.32	(1.27,4.26)
Demeton 566	8	31	0.87	(0.37,2.04)	23	15	4.74	(2.27,9.92)	9	4	5.93	(1.69,20.87)
Mevinphos 480	23	52	1.34	(0.74,2.43)	37	32	3.92	(2.21,6.95)	16	15	3.21	(1.47,6.98)
Phosalone 479	12	23	1.99	(0.91,4.39)	10	9	3.47	(1.31,9.23)	10	8	3.65	(1.34,9.91)
Phorate 478	31	53	2.04	(1.18,3.53)	24	31	2.45	(1.31,4.57)	28	28	3.19	(1.72,5.94)
Parathion 459	34	84	1.43	(0.86,2.37)	35	52	2.29	(1.34,3.91)	50	69	2.43	(1.51,3.91)
Naled 418	36	66	2.16	(1.29,3.61)	47	53	2.99	(1.80,4.95)	35	51	2.16	(1.27, 3.70)
Parathion-methyl 394	20	36	1.81	(0.96,3.42)	20	17	4.47	(2.12,9.41)	10	16	1.68	(0.71,4.00)
Oxydemeton-methyl 382	27	56	1.70	(0.97,2.98)	29	47	2.21	(1.26,3.88)	22	12	5.86	(2.68,12.82)
Malathion 367	52	81	2.16	(1.36,3.43)	44	43	3.16	(1.88,5.32)	25	31	2.69	(1.45,5.01)
Phosmet 335	26	88	1.41	(0.86,2.31)	43	55	2.90	(1.74,4.81)	34	52	1.93	(1.13,3.30)
Azinphos-methyl 314	30	76	1.46	(0.86,2.45)	22	36	2.12	(1.14,3.95)	37	40	2.88	(1.66,4.98)
Merphos 293	27	31	3.13	(1.67,5.79)	16	17	2.72	(1.27,5.83)	8	7	3.73	(1.22,11.42)
Ethion 268	13	25	1.93	(0.90,4.11)	25	25	3.83	(1.98,7.40)	9	9	3.44	(1.26,9.42)
Chlorpyrifos 253	46	88	1.69	(1.06,2.69)	31	57	1.94	(1.12,3.34)	39	64	1.92	(1.15,3.18)
Disulfoton 230	24	45	1.82	(1.01,3.28)	24	26	2.97	(1.55,5.67)	16	15	2.88	(1.32,6.33)
Dimethoate 216	47	100	1.71	(1.08,2.72)	54	62	3.04	(1.89,4.90)	66	85	2.59	(1.66,4.05)
Diazinon 198	47	109	1.47	(0.93,2.32)	37	64	1.93	(1.16,3.21)	58	71	2.61	(1.64,4.16)
Tribufos 190	23	57	1.36	(0.77,2.42)	27	40	2.28	(1.26,4.10)	24	23	3.15	(1.62,6.12)
Trichlorfon 88	15	32	1.55	(0.77,3.12)	19	17	4.24	(2.01,8.95)	6	5	3.35	(0.95,11.78)
Monocrotophos 52	32	51	2.03	(1.18,3.51)	21	25	2.66	(1.36,5.21)	24	15	5.53	(2.62,11.65)

* Adjusted for sex, education, smoking, age, family history of PD, race, and other pesticides.

Note: The following OPs were not included in this analysis due to too few exposed participants at either residences only, workplaces only, or at residences and workplaces: leptophos, sulfotep, phosphamidon, carbophenothion, dicrotophos, TEPP, dioxathion, dialifor, dichlorvos, and bensulide

Table 6.3. Effect estimates for ambient exposures to subsets of organophosphates based on mechanism of toxicity at residences and workplaces during 1974-1999

	Residences only				Workplaces only				Residences and workplaces			
	case	control	Adj OR	95% CI	case	control	Adj OR	95% CI	case	control	Adj OR	95% CI
Not exposed to pesticides	65	220	1.00	reference	65	220	1.00	reference	65	220	1.00	reference
Exposed to cholinesterase inhibiting OPs	75	175	1.45	(0.97,2.16)	39	75	1.79	(1.09,2.94)	165	254	2.24	(1.58,3.19)
Exposed to highly toxic OPs	69	154	1.53	(1.01,2.30)	45	78	1.88	(1.17,3.03)	133	196	2.40	(1.65,3.47)
Exposed to teratogen OPs	64	146	1.54	(1.01,2.34)	53	82	2.11	(1.33,3.34)	99	144	2.41	(1.62,3.58)
Exposed to endocrine disruptor OPs	74	166	1.53	(1.03,2.30)	39	83	1.57	(0.96,2.55)	143	206	2.41	(1.67,3.48)
Exposed to carcinogen OPs	36	78	1.56	(0.95,2.56)	36	46	2.73	(1.59,4.71)	29	29	3.21	(1.75,5.91)
Exposed to mitochondria disruptor OPs	69	138	1.70	(1.13,2.58)	53	84	2.22	(1.41,3.51)	110	168	2.23	(1.52,3.27)

* Adjusted for sex, education, smoking, age, family history of PD, race, and other pesticides.

Note: Number of participants in this table do not add up to the total number of participants because the subsets of OPs are not mutually exclusive and participants that

Table 6.4. Effect estimates for ambient exposures to mitochondrial disrupting organophosphates at residences and workplaces during 1974-1999

	Residences only				Workplaces only				Workplaces and Residences			
	cases	controls	Adj OR*	95% CI	cases	controls	Adj OR*	95% CI	cases	controls	Adj OR*	95% CI
Not exposed to pesticides	65	220	1.00	reference	65	220	1.00	reference	65	220	1.00	reference
Non-mitochondria disrupting OPs												
Exposed to only OPs that don't disrupt mitochondria	18	59	1.09	(0.59,2.01)	15	25	2.06	(0.99,4.29)	9	15	2.07	(0.84,5.06)
Mitochondria disrupting OPs												
Exposed to 1-7 mitochondria disrupting OPs	74	153	1.63	(1.09,2.44)	46	75	2.16	(1.34,3.48)	80	143	1.98	(1.32,2.97)
Exposed to 8-14 mitochondria disrup	-	-			-	-			37	34	3.37	(1.90,5.95)

* Adjusted for sex, education, smoking, age, family history of PD, race, and other pesticides.

** A total of seven different mitochondria inhibiting OPs were inculded in this study, participants who were exposed to the same OP pesticide at workplaces and residences were considered exposed to two pesticides

Note: Number of participants in this table do not add up to the total number of participants because those that were not exposed OPs but to other pesticides were not included in this table, although they were included in the model.

Table 6.5. List of Organophosphate Pesticides Included in this Study

Pesticide	ChemCode	Toxicity	Carcinogen	Cholinesterase inhibitor	Teratogen	Endocrine disruptor	Mitochondria disruptor
profenofos	2042	0	0	1	0	0	0
fenamiphos	1857	1	1	1	0	0	0
dialifor	1799	0	0	1	0	0	0
methamidophos	1697	1	0	1	0	0	0
methidathion	1689	1	1	1	0	0	0
acephate	1685	0	1	1	0	1	0
leptophos	1676	0	0	1	0	0	0
ethephon	1626	0	0	1	0	0	0
TEPP	577	1	0	1	0	0	0
demeton	566	1	0	1	0	0	0
sulfotep	558	1	0	1	0	0	0
phosphamidon	482	1	1	1	0	1	0
mevinphos	480	1	0	1	0	1	1
phosalone	479	0	0	1	0	0	0
phorate	478	1	0	1	0	0	0
parathion	459	1	1	1	0	1	1
naled	418	0	0	1	1	0	0
parathion-methyl	394	1	0	1	0	1	1
oxydemeton-methyl	382	1	0	1	1	1	0
malathion	367	0	1	1	0	1	1
phosmet	335	0	1	1	0	0	0
azinphos-methyl	314	1	0	1	0	0	0
merphos	293	0	0	1	0	0	0
ethion	268	1	0	1	0	0	0
chlorpyrifos	253	0	0	1	0	1	1
disulfoton	230	1	0	1	0	0	0
dimethoate	216	1	1	1	1	1	1
diazinon	198	0	0	1	1	1	0
dioxathion	192	1	0	1	0	0	0
tribufos	190	0	1	1	0	0	0
dichlorvos	187	1	1	1	0	1	1
carbophenothion	110	1	0	1	0	0	0
trichlorfon	88	0	1	1	0	1	0
dicrotophos	72	1	1	1	0	0	0
bensulide	70	0	0	1	0	0	0
monocrotophos	52	1	0	1	0	0	1

toxicity: 1= extreme, high; 0 = moderate, slight

carcinogen: 1= yes, possible; 0= not likely, not listed

Chapter VII: Overall Summary and Discussion of Research Findings

Pesticide exposures have been demonstrated to be implicated in PD etiology in several studies. However their role is complex due to the fact that pesticides are often applied agriculturally in conjunction or in close succession with each other. Previous studies have faced many difficulties in exploring the relationship between pesticide exposure and PD due to relying on self-reported exposures prone to recall bias, being unable to identify exposures to specific pesticides, and not having a large enough study population exposed to pesticides to detect associations between specific pesticides and PD. These limitations in previous studies have led to much heterogeneity in their published results. Thus, we aim to improve upon previous methods by using a GIS-based approach that utilizes geocoded address data as well as historical land use and pesticide application records to explore the relationship between ambient pesticide exposure and PD.

First, we sought to assess whether the dithiocarbamates, maneb and ziram, confer increased risks of PD when applied with or without paraquat at residences and workplaces. Our results suggest that exposure to maneb, ziram, and paraquat may act together to increase risk of PD more strongly than exposure to other combinations of these three pesticides. Analysis of exposure time window show that ambient exposure at workplaces in the later time window is associated with increased PD risk, whereas only ambient exposure in the early time window at residences was associated with PD risk. This suggests that ambient exposures at workplaces may be more intense so that later exposures in life may also contribute to PD risk. Younger participants, 60 years or younger, also experienced greater risks of PD when exposed to combinations of maneb, ziram, and paraquat compared to participants over 60 years old.

Second, we explored the relationship between exposures to paraquat and three pesticide classes, organophosphate, organochlorine, and dithiocarbamate, and increased risk of PD. We also assessed whether pesticide exposure duration and intensity play a role in the etiology of PD. We found that paraquat, organophosphates, organochlorines, and dithiocarbamates were associated with increased risks of PD, after adjusting for covariates. However, when we adjusted for other pesticides, only organophosphates remained associated with increased PD risk. This phenomenon may be due to the fact that the majority of our study participants were exposed to organophosphates and also co-exposed to the other pesticide classes so that organophosphate exposure may be a proxy for other pesticide exposure. However, it cannot be ruled out that organophosphates have an independent effect on PD risk. Our results also suggest that duration of exposure and being co-exposed to multiple pesticides within the same year contributed to elevated PD risk.

Third, we assessed whether exposure to individual organophosphate pesticides and groups of organophosphates based on their mechanisms of toxicity impact PD risk. Our results suggest that all of the organophosphates included in our study conferred an increased risk of PD. We also show that exposure to an increasing number of organophosphates is associated with elevated PD risk.

Our data provides a unique opportunity to study the relationship between ambient exposures to specific pesticides and PD risk. Our results provide additional support that pesticides are implicated in the etiology of PD in a human population. Specifically, pesticides may act together to increase the risk through mechanisms of toxicity demonstrated by animal models and cell cultures. We also demonstrate that participants in our study are co-exposed to multiple different pesticides and pesticide classes. This underscores the need for future studies to

account for these co-exposures through assessing pesticides in combination with each other. Ambient pesticide exposures at workplaces also appear to be generally associated with greater risk of PD than ambient exposures at residences.

7.1. Strengths and Weaknesses

A strength of our study is that we were able to estimate ambient pesticide exposures through our GIS-based model. Previous studies that relied on self-reported exposure can only measure pesticide exposure that study participants were aware of. However, study participants who live in areas that are heavily agricultural will often be ambiently exposed to a plethora of pesticides applied nearby their residences or workplaces. By not using self-reported exposures we were able to avoid recall bias of exposure and take into consideration the exact type, amount, location, and timing of pesticide exposure, which is a vast improvement over other studies. Another strength of this study is that movement disorder specialists examined patients multiple times to confirm diagnoses, reducing disease misclassification.

A limitation is that our data cannot be considered a quantitative measure of exposure because the derived poundage of active ingredient per acre applied does not translate easily into a measure of human neurotoxicity across pesticides or pesticide classes. Although we were able to avoid recall bias in exposure assessment, we still relied on participants to report their address information accurately in order to precisely geocode these addresses. We found that cases and controls reported their address information with similar accuracy, suggesting that geocoding precision is not likely to contribute to the difference in the effect estimates of the cases and controls.

7.2. Public Health Implications

Although this study focuses of PD, a myriad of other diseases also result from exposure to toxic chemicals in the environment. Unfortunately those who live and work in rural areas are among the most vulnerable due to their greater exposure to agriculturally applied pesticides coupled with weaker health care infrastructure. However, the farming industry located in the Central Valley of California that feeds the nation with its produce relies heavily on pesticides to maintain and increase their harvests. The current study explores the risks associated with exposure to certain pesticides and combinations of those pesticides in an attempt to not only protect the vulnerable population, but also to highlight which chemicals are not as toxic to humans and whose use should be promoted. Research on harmful chemicals will in turn promote adherence to higher safety standards. Therefore it is of the utmost importance to continue to refine and innovate new methods and technologies to estimate and model pesticide exposure. More precise exposure assessment and more complete understanding of the etiology of PD are steps that will lead us to more effective prevention of PD and preservation of the environment.

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