ENVIRONMENTAL, OCCUPATIONAL, AND PERSONAL LIFESTYLE RISK FACTORS FOR AMYOTROPHIC LATERAL SCLEROSIS: A CASE-CONTROL STUDY

by

Angela Marie Malek

BA, University of South Carolina, 2003

MPH, University of Pittsburgh, 2006

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This dissertation was presented

by

Angela M. Malek

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and approved by

Dissertation Advisor:
Evelyn O. Talbott, DrPH, MPH
Professor
Department of Epidemiology
Graduate School of Public Health, University of Pittsburgh

Committee Member:
Aaron Barchowsky, PhD
Professor
Department of Environmental and Occupational Health
Graduate School of Public Health, University of Pittsburgh

Committee Member:
Robert Bowser, PhD
Associate Professor
Department of Pathology
School of Medicine, University of Pittsburgh

Committee Member:
David Lacomis, MD
Professor
Department of Neurology
School of Medicine, University of Pittsburgh

Committee Member:
Ada Youk, PhD
Assistant Professor
Department of Biostatistics, Department of Epidemiology
Graduate School of Public Health, University of Pittsburgh

ENVIRONMENTAL, OCCUPATIONAL, AND PERSONAL LIFESTYLE RISK FACTORS FOR AMYOTROPHIC LATERAL SCLEROSIS: A CASE-CONTROL STUDY

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ALS or Lou Gehrig's disease is the most common motor neuron disease (MND) in adults. Male sex and age are the only known risk factors for ALS; however, an environmental etiology for ALS has been supported by epidemiologic studies and is suggested by the gender discrepancy. The objectives of this dissertation are to: 1) explore the relationship between pesticide exposure and risk of ALS through meta-analysis; 2) validate an ALS questionnaire to measure important risk factors in an ALS specific population; 3) examine the association of sociodemographic characteristics, personal risk factors, and occupational and environmental exposures and ALS; and to 4) evaluate the association of hazardous air toxics and risk of ALS by linkage to an existing database. Through this dissertation, a case-control study was conducted of 66 ALS cases and 66 matched controls from 2008-2011 to investigate risk factors for ALS. Existing data from the Environmental Protection Agency National-Scale Air Toxics Assessment was also analyzed in relation to residence of cases and controls.

The results showed exposure to pesticides, solvents, and metals may increase the risk of ALS. In conclusion, several environmental and occupational exposures are suggested to play a role in the risk of ALS as well as other neurological diseases. This dissertation is of public health significance as it fills an important gap in ALS research and proposes a new direction of investigation for ALS, that of hazardous air pollutants.

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PREFACE

I would like to extend my sincere appreciation to my advisor, Dr. Evelyn Talbott, who has guided me with her experience, wisdom, and enthusiasm throughout every step of the PhD journey. Dr. Talbott's patience, insight, and endless encouragement are most appreciated. I would also like to express my gratitude for the support, guidance, and helpful feedback provided by each of my committee members. Without such a wonderful advisor and committee none of this would have been possible.

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1.0 INTRODUCTION

Amyotrophic lateral sclerosis (ALS) or Lou Gehrig's disease is the most common motor neuron disease (MND) in adults and is characterized by progressive degeneration of both the upper and lower motor neurons in the brain and spinal cord International Classification of Diseases, 9th edition (ICD-9) code for ALS is 335.20 and the ICD-10 code, G12.2. The global incidence of ALS is about 1-3 cases per 100,000 annually, and the prevalence is approximately 6 cases per 100,000 (Mitchell and Borasio 2007; Alliance 2009; Migliore and Coppede 2009). There are approximately 5,600 new cases of ALS diagnosed in the U.S. each year, and the prevalence of ALS is 20,000 to 30,000 persons annually (Association 2007). ALS is a rare disease that usually increases in incidence in those over fifty years of age until the age of 75 (Kamel, Umbach et al. 2005; Alliance 2009; Migliore and Coppede 2009). Men develop ALS at 1.5 times the rate of women although the inequality seems to balance out among the two sexes in older age (Kamel, Umbach et al. 2005). In general, trends over time indicate that mortality from ALS has been increasing in the U.S., Israel, Europe, and Japan (Kelly 2001). Overall, incidence of ALS is similar among developed countries. Any minor deviations are presumed to be due to the quality of data.

ALS is a degenerative disease involving muscle weakness and atrophy that later affects respiration, ultimately resulting in death (Borasio and Miller 2001). After onset of ALS has occurred, the median survival is 2-4 years (Borasio and Miller 2001). Rilutek is currently the

only medication available for treatment of ALS. It extends life by 2-3 months. No other treatment exists to treat or prevent this devastating disease.

An environmental etiology for ALS has been supported by epidemiologic studies (Nelson 1995; Strong and Rosenfeld 2003). The gender discrepancy also points to a possible environmental association. Western Pennsylvania (W. Pa) is known for its long history of steel making. In the late 1960's, the industrial labor force of approximately 365,000 steel workers was at its peak. This included employment of both men and women. Furthermore, the majority of W. Pa residents often remain in the area throughout their lifetime, providing an ideal research setting for studying the effects of environmental exposures and the development of ALS. Many occupational and personal risk factors have been investigated over the years, some of which have produced conflicting results. In conclusion, many people around the world are affected by this debilitating disease, while possible causes of sporadic ALS remain unknown.

1.1 HISTORY AND DEFINITION

ALS was first discovered by French neurologist, Jean Marie Charcot, in the late 1800's (Association 2007). ALS is also referred to as Lou Gehrig's disease because of the great New York baseball player, Lou Gehrig, who retired from baseball in 1939 due to health problems and was diagnosed with the disease months later (Association 2007). The term, amyotrophic lateral sclerosis, comes from the Greek origin, meaning the thinning and wasting of muscles (amyotrophy) because of a lack of innervation. Lateral sclerosis refers to hardening of the lateral corticospinal tracts in the spinal cord due to loss of upper motor neuron projections. The inability of messages to be sent to the muscles as a result of neuron death precipitates the

impending muscle weakness and progression of associated symptoms. There are two categories of ALS, sporadic and familial. Both forms of ALS usually exhibit similar symptoms and disease progression. This study and report will focus on sporadic ALS exclusively.

The term motor neuron disease (MND) refers to a group of diseases in which the motor neurons degenerate, and includes a number of diseases such as ALS, spinal muscular amyotrophy (SMA), postpolio syndrome, primary lateral sclerosis (PLS), progressive bulbar palsy (PBP), pseudobulbar palsy, and progressive muscular atrophy (PMA). Most, but not all, of those diagnosed with PLS, PBP, pseudobulbar palsy, or PMA are subsequently diagnosed with ALS.

Motor neurons control voluntary muscle activity. In a healthy individual, upper motor neurons found in the precentral gyrus of the cerebral cortex, transmit signals to the lower motor neurons of the spinal cord and brain stem via corticospinal and corticobulbar pathways. Signals are then sent to particular skeletal and bulbar muscles in the body. In someone affected with ALS, these signals are interrupted as the neurons are progressively degenerating and dying. Therefore, the brain loses its ability to initiate and control muscle movement. The loss of spinal cord and brainstem motor neurons results in muscle wasting, spasticity, fasciculations or uncontrollable twitching, and later paralysis. Spasticity occurs as a result of the loss of brain upper motor neurons and their inhibitory signals that project onto lower motor neurons. This scenario leads to loss of control of muscle tone with overactivity and hyperreflexia. Gradual muscle weakness may manifest as difficulties in speaking, swallowing, walking, and breathing. In some cases, cognitive changes similar to those seen in frontotemporal dementias occur when the prefrontal motor neurons are affected (Ludolph, Langen et al. 1992). Milder frontal dysfunction (dysexecutive syndrome) is fairly common.

1.2 GENETICS OF ALS

1.2.1 Genetics of Sporadic ALS

Sporadic ALS (SALS) is the most common form of ALS accounting for approximately 90-95% of all cases (Migliore and Coppede 2009). The disease seems to occur randomly as no other family members will have had the disease. It can develop in anyone, anywhere, but it is uncommon prior to age 40. The etiology and methodology of SALS is not known. Although this form of the disease is not hereditary, the disease is complex and it's believed that genetic susceptibility to environmental risk factors and possibly other genes plays a role.

The gene on chromosome 21q21 (ALS1) responsible for regulating the protein, copper-zinc superoxide dismutase 1 (*SOD-1*) has been found to be associated with a small proportion of SALS though two mutations, I113T and D90A (Beal 2005). These genes are both considered to be expressed by low penetrance. Because of this, it may be difficult to differentiate environmental factors from genetic factors.

Survival motor neuron (*SMN*) and neuronal apoptosis inhibitory protein (*NAIP*) are involved in the development of spinal muscular atrophies and have been studied as possible susceptibility genes. The *SMN* and *NAIP* genes have been found to be mapped to a modifier gene in an animal model of *SOD-1* (Nelson 2004). ALS prognosis is influenced by the *SMN1* gene (Nelson 2004). In addition, an ALS phenotype was discovered to be related to the number of SMN1 copies present in families where SMA or ALS was diagnosed (Nelson 2004). A prognostic factor in SALS is homozygous deletion of the *SMN2* gene although ALS was not shown to develop on account of spinal muscular deletions (Nelson 2004). Other possible genes that may be risk factors for SALS include those coding for the *haemocromatosis* gene (HFE) and neurofilaments

(*NEFL, NEFM, NEFH)* (Migliore and Coppede 2009). An increase of copy numbers of *SMN* genes and the *HFE* 63D variant was reported to be involved in ALS risk according to a pooled analyis (Migliore and Coppede 2009). A polymorphism in the *dipeptidyl-peptidase 6* gene has also been discovered to be linked to ALS susceptibility (Migliore and Coppede 2009).

Susceptibiltiy to sporadic ALS has als been linked to genetic modifications in the paraoxonase gene cluster (PON1) (Landers, Shi et al. 2008). Landers et al. evaluated 597 case and 692 control samples for 20 SNPs across the paraoxonase gener cluster to determine if there was any potential relationship with SALS, and found that two SNPs [rs987539 (p=0.000647) and rs2074351 (p=0.000787)] within the gene cluster were associated with SALS susceptibility (Landers, Shi et al. 2008). No relationship was found with regard to disease survival, age of onset, or site of onset for the 20 SNPs and SALS. SALS was however, found to be associated with a 5-SNP haplotype (p=0.0000275) (Landers, Shi et al. 2008).

TAR DNA binding protein, also known as TDP-43 or TARDBP, has been shown to build up in the nerve cells in patients with MND (Sreedharan, Blair et al. 2008). SALS and FALS have both been linked to mutations in the *TARDBP* gene (Daoud, Valdmanis et al. 2009). This gene was found to be responsible for apoptosis of nerve cells. Researchers are still unsure as to the specific role of the gene in the nervous system and more research must be conducted regarding this new area of ALS pathogenesis.

It is hypothesized that SALS and dementia have a common genetic susceptibility based on a small number of studies and one twin study in the United Kingdom that found an association (Beghi, Mennini et al. 2007). Parkinson's disease may also share a genetic susceptibility with SALS (Beghi, Mennini et al. 2007). A case-control study of 185 Polish patients with definite or probable SALS and 437 age- and sex-matched controls was conducted bu Slowik et al. in 2006

to evaluate the relationship between amino acid variants (PON1 and PON2) and ALS (Slowik, Tomik et al. 2006). An association was found between ALS and the C allele of the PON2 C311S polymorhism (Slowik, Tomik et al. 2006). This was true for both dominant and additive logistic regression models. Likewise, in dominant, additive, and recessive models, the R allele of the PON1 Q192R polymorphism was linked to SALS (Slowik, Tomik et al. 2006). Compared to controls, a greater proportion of the cases had the R-C haplotype (OR=3.44; 95% CI=1.55, 7.62; p=0.002) (Slowik, Tomik et al. 2006).

Oxidative stress markers have been suspected to play a role on the etiology of SALS. A cross-sectional pilot study of 50 SALS patients and 46 controls conducted by Mitsumoto et al. found SALS patients had significantly higher levels of two oxidative stress biomarkers (8-oxodG and IsoP) compared to compared to controls (Mitsumoto, Santella et al. 2008). After adjusting for gender and age, the differences remained (Mitsumoto, Santella et al. 2008). A positive correlation was shown between the two markers (Mitsumoto, Santella et al. 2008).

APEX1 and *hoGG1* are both involved in the defense of oxidative stress and as such are implicated in SALS as candidate genes (Migliore and Coppede 2009). Both are DNA repair genes. Studies have produced inconsistent findings related to the risk of SALS and the *APEX1* D148E polymorphism (Migliore and Coppede 2009). Recently, Coppede et al. conducted a case-control study of 134 patients with SALS and 129 matched controls and reported no association (Migliore and Coppede 2009). The publication is currently in press. Another DNA repair gene, *hOGG1 Ser326Cys polymorphism*, has been studied by Coppede et al. in a case-control study of similar numbers (Migliore and Coppede 2009). This study found a relationship between risk of SALS and male gender (Migliore and Coppede 2009).

Other likely SALS risk factors include two angiogenesis factors, ANG and VEGF. Risk of SALS in Irish and Scottish populations was found to be related to the *ANG* G110G polymorphism although this finding has not yet been reproduced in other populations (Migliore and Coppede 2009). Results, however, have been dubious or negative for any link of SALS to the *VEGF* gene (Migliore and Coppede 2009).

Motor neurons may be affected through glutamate toxicity, oxidative damage, accumulation of intracellular aggregates, glial cell pathway, mitochondrial dysfunction, defects in axonal transport, growth factor deficiency, and aberrant RNA metabolism. Disease onset and progression may be influenced by a combination of these various processes. In summary, at least ten mutations have been associated with SALS. Four oxidative stress biomarkers and two angiogenesis factors have also been linked to SALS development. The presence of these risk factors only accounts for a fraction of the total number of SALS cases as this type of the disease is primarily sporadic by nature. As more genetic research is conducted, it is likely that more mutations will be found to be associated with SALS. Many studies have been carried out to evaluate risk factors for developing SALS although future research is needed to further elucidate potential causes of SALS.

1.2.2 Genetics of Familial ALS

Familial ALS (FALS) is the inherited form of ALS and accounts for 5-10% of all ALS cases (Migliore and Coppede 2009). A genetic abnormality or mutation is the cause of FALS. Familial ALS is defined by having one or more family member affected by ALS, and is characterized by a 15-20 year earlier mean age of onset compared to sporadic ALS (Nelson 2004). The number of family members with FALS varies by family. When many individuals in

a family are affected by FALS, they are said to have causative genes. A causative gene means that excluding other factors, having the FALS gene alone is sufficient in producing ALS. Families with only a small number of affected individuals may have either susceptibility or causative genes. Inheritence of ALS can follow a Mendelian pattern but can also be familial without a Mendelian pattern of disease inheritence. In general, offspring of FALS parents have a 50% chance of developing the disease because most cases of FALS are inherited in an autosomal dominant manner. The disease may also be inherited in an autosomal recessive manner or X-linked recessive method although this is rare. The majority of offspring who inherit the abnormal gene will develop ALS, but they can also be a carrier of the gene by passing it on to the next generation without developing the disease themselves.

In 1993, the gene on chromosome 21q21 (ALS1) responsible for regulating the protein, copper-zinc superoxide dismutase 1 (*SOD-1*), was discovered (Miller 2005). More than 100 alleles have been identified with only two resulting in autosomal recessive inheritence (D90A and L84F); the remainder are autosomal dominant (Nelson 2004; Murray 2006). Twenty percent of FALS patients (1-2% of all ALS patients) have the mutated *SOD-1* gene (Miller 2005; Association 2007). *SOD-1*'s role is to remove free radicals, which are the waste products of cell metabolism (Miller 2005). Routine testing for *SOD-1* has become available through commercial labs but it's not recommended for the vast majority of patients because of the very low sensitivity of the test. Despite having ALS, the test identifies 98-99% of patients as negative for the gene in the general ALS population.

Scientists are still searching for abnormal genes that might be associated with the remaining 80% of FALS cases. A number of chromosomes have been linked to FALS. These include dominant mutations found on chromosome 16 and the X chromosome, and recessive mutations

found on chromosomes 2 and 15 (Association 2007). The recessive form of FALS is rare and the disease has a long duration and an early onset. It's been discovered that 10-15% of FALS may have symptoms of frontotemporal (FTD) dementia (Association 2007). A place on chromosome 9 has been linked to this condition (Association 2007).

Neuronal degeneration in MND has been thought to be linked to impaired axonal transport (Puls, Jonnakuty et al. 2003). In 2003, a study of a family with a slowly progressive, autosomal dominant form of lower MND disease without sensory symptoms discovered that MND in humans can occur as a result of dynactin-mediated (*DCTN1)* transport dysfunction on chromosome 2p13 (Puls, Jonnakuty et al. 2003; Migliore and Coppede 2009). Onset occurred in early adulthood and the family experienced particular symptoms such as vocal cord paralysis, breathing problems, and lower limb muscle weakness and atrophy related to the *dynactin* gene mutation (Migliore and Coppede 2009). As a result of animal studies, we know that the integrity of synapses at the neuromuscular junctions are supported by dynactin.

In 2009, two studies were conducted on *ELP3*, the RNA polymerase II component and its role in neuronal degeneration and axonal biology of ALS (Simpson, Lemmens et al. 2009). The investigators reported a link betweeb ALS and allelic variants of *ELP3* in a sample of three human populations (N=1483) (p=1.96 x 10^{-9}) (Simpson, Lemmens et al. 2009). Two different loss of function mutations for genes involved in neuronal communication and survival were discovered in *ELP3* (R475K and R456K) in *Drosophila* (fruit flies) (Simpson, Lemmens et al. 2009). Dose-dependent motor axonal abnormalities were found in zebra fish embryos as a result of knock down of *ELP3* protein levels [Pearson correlation: -0.49, p=1.83 x 10^{-12} (start codon morpholino)] (Simpson, Lemmens et al. 2009). Reduced brain *ELP3* expression was correlated in humans with *ELP3* genotypes (p=0.01) (Simpson, Lemmens et al. 2009).

The gene (ALS2) associated with autosomal recessive ALS (RFALS) was found on chromosome 2q33 (Kanekura, Hashimoto et al. 2004). It also includes a juvenille form of disease with onset before age 25. RFALS has been reported in Tunisia and in areas of high ancestry but in general, this form of ALS is rare (Migliore and Coppede 2009). The gene affects North African and Middle Eastern inbred families (Kanekura, Hashimoto et al. 2004). Alsin is the protein programmed by ALS2 and is associated with the supression of motoneuronal death induced by FSOD1 through the Rac1/phosphatidylinositol-3 kinase/Akt3 pathway (Matsuoka and Nishimoto 2005). There are two splicing variants of ALS2, alsin long form (LF) and alsin short form (SF). The development of autosomal dominant ALS caused by the SOD-1 gene, was prevented with the protection of motor neuron cells as a result of alsin LF expression (Kanekura, Hashimoto et al. 2004). According to Kanekura et al., "Alson LF belongs to the family of the guanine nucleotide exhanging factor (GEF) for small GTPases" (Kanekura, Hashimoto et al. 2004). An anti-Alzheimer's neutrophic factor has demonstrated that the Ca^{2+}/calmodulin-dependent protein kinase IV pathway is (Matsuoka and Nishimoto 2005).

An autosomal recessive juvenille-onset form of ALS in the teen years can occur as a result of a mutation of the *senataxin* protein (*SETX)* coded for by a gene on chromosome 9q34 (ALS4) in seven Brazilian families (Alliance 2009; Migliore and Coppede 2009). *SETX* helps to protect against oxidative DNA damage. The disease is expressed by a dominant inheritence pattern meaning it is inherited from one parent. The vesicle-associated membrane protein/synaptobrevin-associated membrane protein B gene (VAPB) on chromosome 20q13.3 (ALS8) can have a mutation in its gene leading to a dominant inherited pattern of adult-onset ALS (Alliance 2009; Migliore and Coppede 2009). The same is true when a mutation occurs on the *angiogenin* protein coded for by a gene on chromosome 14 (Alliance 2009).

A missense mutation found on chromosome 16p11.2 of the fused in sarcoma/translated in liposarcoma (FUS/TLS) gene is linked to a proportion of FALS cases, also known as ALS6 (Chio, Restagno et al. 2009; Vance, Rogelj et al. 2009). Homology has been observed between *FUS and TARDBP* (Vance, Rogelj et al. 2009). This implies the possibility that motor neuron degeneration may involve a shared method by the two genes.

Over thirty years ago, the association of ALS with frontotemporal dementia (FTD) that is frequently seen with symptoms of Parkinsonism was identifed (Migliore and Coppede 2009). This atypical form of ALS is associated with chromosome 9q21-q22 (Migliore and Coppede 2009). ALS and FTD has been recognized in a Swedish family on chromosome 9pq21.3-p13.3 (Migliore and Coppede 2009). ALS with Parkinsonism and FTD (ALS and FTDP) was identified on chromosome 17q21 through mutations on the microtubular associated protein tau (*MAPT)* (Migliore and Coppede 2009).

Van de Giessen et al. conducted a study of 251 FALS patients to investigate the relationship between glutathione S-transferase omega 1 and 2 (GSTO1 and 2) and ALS pathogenesis (van de Giessen, Fogh et al. 2008). Age of onset of FALS was linked to some SNPs in Swedish patients (p=0.003-0.048) (van de Giessen, Fogh et al. 2008). No relationship was found between age of onset and survival in British and Australian ALS patients.

A case-control study conducted in India by Babu et al. in 2008 of 22 ALS patients and 20 controls found ALS patients had increased inflamatory markers compared to controls (Babu, Kumar et al. 2008). Specifically, interferon-gamma (IFN-gamma) levels and serum tumor necrosis factor-alpha (TNF-alpha) levels were higher in ALS patients (p<0.001) (Babu, Kumar et al. 2008). Compared to controls, ALS patients also had elevated levels of nitric oxide (p<0.05) (Babu, Kumar et al. 2008). Proinflammatory molecule levels and duration of ALS were

correlated (Babu, Kumar et al. 2008). Increased levels of TNF-alpha are cytotoxic and may contribute to the development of ALS.

In 2009, a review article by Rothstein stated that in persons with SALS and FALS mutations and pathology related to the TDP-43 gene and protein may be more common than SOD1 mutations (Rothstein 2009).

Overall, a number of genes have been associated with FALS over the years. As mentioned earlier, twenty percent of FALS cases are associated with mutations in the SOD-1 gene. Some of the remaining eighty percent of FALS cases have been linked to six chromosomes, six mutations, and an increase in two inflammatory markers. Researchers will contintue to search for additional genes that may be linked to the development of FALS.

1.2.3 Genetics of Western Pacific ALS

A variant of ALS was discovered in western Pacific countries among the Chamorros or indigenous people of Guam (Nelson 1995). This form of the disease presents similarly to sporadic ALS, but some patients may also have parkinsonism and/or dementia. The parkinsonism-dementia complex (PDC) is a neurodegenerative and fatal disease, similar to ALS . The western Pacific variant of ALS/PCD is the largest and most popular cluster due to the excess number of cases (Nelson 2004).

The risk of ALS was found to be about 50 times higher than expected in the early-1950's in the areas of Guam, the other Mariana islands, the Kii Peninsula of Japan, and Kepi on the southern coast of western New Guinea (Nelson 1995; Beghi, Mennini et al. 2007). Although the etiology of this form of ALS is not known, several hypotheses have been investigated such as a genetic origin, differences among ethnic groups, environmental factors in soil or water,

migratory bird exposure, nutrition, and the nut of the *Cycas circinalis* tree (Beghi, Mennini et al. 2007). Over the past 40 years, ALS incidence in Guam and South West Papau has continued to decrease (Beghi, Mennini et al. 2007). Incidence of ALS in the Kii peninsula of Japan has also decreased although the decline has been slower in men (Beghi, Mennini et al. 2007). Genetics, westernization, socioeconomic, ecologic, and ethnic factors are all thought to play a role in the reduction of incidence in western Pacific countries (Beghi, Mennini et al. 2007).

1.3 EPIDEMIOLOGY OF ALS

ALS is a rare disease with a global incidence of 1-3 cases per 100,000 population and an estimated prevalence of 6 cases per 100,000 (Kamel, Umbach et al. 2005; Mitchell and Borasio 2007; Alliance 2009; Migliore and Coppede 2009). Incidence increases between persons over 50 years of age to 75 years (Migliore and Coppede 2009). European registries have recently reported the incidence rate to be between 1.7 and 2.5 cases per 100,000 population (Beghi, Mennini et al. 2007). Every day, 328 new cases are diagnosed worldwide (Alliance 2009). Annually, the worldwide incidence of ALS is 120,000 cases (Alliance 2009). The incidence of ALS has been found to be relatively homogeneous in industrialized European nations when comparable diagnostic criteria and case ascertainment standards apply (Beghi, Mennini et al. 2007). In places other than European populations, ALS clinical characteristics, incidence, and prevalence are not well known, however, it has been suggested that ALS is less common among African populations (Cronin, Hardiman et al. 2007). In general, trends over time indicate that mortality from ALS has been increasing in the U.S., Israel, Europe, and Japan (Kelly 2001). The age decades of the 50's through 90's have experienced this increase in ALS-related mortality

(Kelly 2001). Reasons for the increase in mortality may include better diagnosis of ALS in the elderly, a real rise in incidence of ALS, and an aging population as life expectancy increases over time. As people live longer, they are more likely to develop age-related diseases, but if that is not the case, ALS may instead develop. ALS cases are included in the classification of MND.

Each year in the United States, there are approximately 5,600 new patients diangosed with ALS or 15 patients daily (Association 2007). The U.S. prevalence of ALS is around 20,000 to 30,000 persons annually (Miller 2005; Association 2007). Age-adjusted mortality rates for MND from 1969-1998 indicate an increasing trend in mortality for men, women and both sexes combined (ATSDR 2003). This increase may be due to more accurate detection or may be a real increase. Diagnosis of ALS most often occurs beween the ages of 40 to 70 with the average age being 55 years. The incidence of ALS increases with age. As the population ages, ALS incidence is expected to rise. Age of disease onset, however, ranges widely from younger patients in their early 20's to older patients in their 90's. Of those diagnosed, half live 3 years or longer following diagnosis. Twenty percent live 5 years or longer, and a small proportion of nearly 10% survive for a period longer than 10 years (Association 2007).

A case-series conducted by Noonan et al. in 2005 compared geographical differen ces in ALS throughout the United States (Noonan, White et al. 2005). The divisions were previously determined by a multiple sclerosis study published in 1979 (Noonan, White et al. 2005). A southeast to northwest gradient of annual age-adjusted MND mortality rates was reported (Noonan, White et al. 2005). Only northern California (Middle West area) was not consistent with the trend (Noonan, White et al. 2005). Examination of previous decades, (1969-1978) and (1979-1988), also found similar results (Noonan, White et al. 2005). The area with the highest mortality rate was the North West area (2.2 per 100,000) (Noonan, White et al. 2005). The

North West area mortality rate was significantly greater than that of the South, East, and Middle Central areas (p <0.05, each comparision) (Noonan, White et al. 2005).

Diagnosis of ALS is usually made twelve months after initial clinical symptoms of disease appear (Nelson 2004). Since muscle weakness in ALS is a gradual progression, the disease must be biologically active well before clinical symptoms actually appear. When one-half of the motor neurons are affected, disease can be clinically identified . Three years following diagnosis is the average lifespan for an ALS patient, around which the majority (90%) of the impaired anterior horn cells have degenerated (Nelson 2004). This is important because researchers believe clinical onset may be preceded by biological initiation of ALS by up to three years . As such, it is recommended that case-control studies assess relevant risk factors well before the pre-clinical disease period which may include 1-5 years before diagnosis has been made (Nelson 2004).

1.3.1 Race/Ethnicity and Gender Variation

ALS is more common among men compared to women with a ratio of 3:2 (Kamel, Umbach et al. 2005; Association 2007; Migliore and Coppede 2009). Men have also been found to develop ALS at a younger age than women, so women may be somehow protected from the disease (Miller 2005). This may be due to environmental reasons or a possible protective effect of estrogen. Incidence of ALS becomes more equal among men and women as age increases and after menopause the ratio becomes 1:1 (Association 2007; Migliore and Coppede 2009). ALS of the lower motor neuron variants occurs tenfold in men compared to women (Migliore and Coppede 2009).

The majority of ALS patients studied have been of Caucasian or White race because research is lacking on many other racial/ethnic groups. In 1991, Kurtzke et al. found that compared to other racial groups, Caucasians had a higher incidence of ALS (Kurtzke 1991). Similar ALS incidence rates were found for African-Americans, Caucasians, and Hispanics in a 1991 study in Harris County, Texas (Annegers, Appel et al. 1991). Noonan et al examined age-adjusted MND mortality rates from 1969-1998 by gender and race (Noonan, White et al. 2005). An increase in age-adjusted MND mortality rates was seen for all groups although in the mid-1980's, the rates for men decreased or stayed the same (Noonan, White et al. 2005). In the early 1990's, African-American men and women experienced a slight decrease in MND mortality (Noonan, White et al. 2005). Only White women had a steady increase in mortality between 1969-1998 (Noonan, White et al. 2005). This may indicate better reporting or possibly a new technique that makes for more sensitive and earlier detection. There may also be a real increase in MND among women.

1.4 SYMPTOMS

Symptoms of ALS often vary in the early stages of disease. Atrophy of the muscles also known as amyotrophy, indicates abnormalities in lower motor neurons. Muscle weakness and atrophy present after 50% of motor neurons are lost (Nelson 2004). In the early stages of disease, localized weakness is usually experienced in one area of the body, although it may occur in multiple parts of the body, such as a hand or foot. Dropping things, tripping, foot slapping, abnormal fatigue of the arms or legs, muscle cramps, muscle twitches, slurred or slowed speech, and uncontrollable periods of laughing or crying are some of the early symptoms often experienced in ALS (Miller 2005; Association 2007). One may have difficulty with everyday

activities such as dressing, washing, opening jars, turning keys, or lifting things if the hands are affected; whereas walking may prove challenging if one's feet are affected. When the upper motor neurons are affected at an early sakwardtage, as in *primary lateral sclerosis*, different symptoms are experienced than those resulting when the lower motor neurons are initially affected. For example, when speech and swallowing have been affected, speech may be slow and slurred. If the hand is affected, slowness, awkwardness, and clumsiness of function may result. This can be seen in a handshake where the person with ALS would return the shake slowly and not as automatic as usual.

The neurological regions that can be affected include: the bulbar, cervical, lumbar, and thoracic (trunk). The clinical characteristics of bulbar-onset patients include difficulty swallowing known as dysphagia, slurring of speech known as dysarthia, or a combination of both symptoms (Mitchell and Borasio 2007). The lower motor neurons, upper motor neurons, or both areas can be affected in the bulbar-onset form of ALS (Mitchell and Borasio 2007). The lower motor neuron abnormality known as bulbar palsy, affects the bulbar muscles resulting in slurred speech, difficulty swallowing, facial weakness, and wasting of the tongue. Pseudobulbar palsy is an upper motor neuron abnormality that affects speech and swallowing, and is associated with a brisk jaw jerk and emotional lability (pseudobulbar affect). Emotional lability is described as excessive laughing or crying in the absence of happy or sad feelings.

With cervical-onset, many different symptoms may be experienced. These can include: wasting of the arm along with brisk refluxes, proximal weakness that may affect shoulder abduction related tasks (brushing hair), and distal weakness (Mitchell and Borasio 2007). In lumbar-onset, lower motor neuron symptoms are seen such as tripping or foot drop or problems using the stairs (Mitchell and Borasio 2007).

It is rare that a person with ALS experiences breathing difficulties as his/her initial symptom of ALS. This usually occurs later as a consequence of loss of motor neurons in the cervical spinal cord that project to the phrenic nerves and diaphragm and is due to involvement of accessory muscles and breathing. Breathing difficulties tend to present when the patient is lying down since there is no assistance in lowering the diaphragm by gravity. Patients may also present with problems sleeping or with shortness of breath from physical exertion. Another common symptom of ALS is weight loss. This can result from amyotrophy, reduced caloric intake, and an increase in metabolism. Cramping in muscles where atrophy has occurred is often seen early in the course of the disease. The majority of ALS patients experience fasciculations or muscle twitches, but this is also common in the general population so it is not a symptom specific to ALS. These are not painful, have no clinical significance with regard to disease progression, and aren't treatable, although patients may express concern especially since they are a visible symptom. All of the symptoms previously described can be treated supportively except for fasciculations, which aren't painful.

As the disease progresses and through paralysis of the trunk and diaphragm, breathing becomes more complicated and ventilatory support may be necessary. In ALS, the mind and one's senses (i.e. sight, smell, touch, etc.) usually remain intact (Miller 2005), although mild congnitive dysfunction is common (Miller 2005).

1.5 DIAGNOSIS

As mentioned previously, ALS diagnosis usually takes place one year after onset of the first clinical symptoms of disease. Diagnosis of ALS can be difficult as a specific diagnostic test or

procedure is lacking for the disease. Certain infectious diseases such as human T-cell leukemia virus (HTLV), HIV, and Lyme disease as well as the following neurological disorders: post-polio syndrome, MS, spinal muscular atrophy, and multifocal motor neuropathy may cause symptoms analogous to those seen in ALS. Diagnosis often occurs through the use of various diagnostic tests at the discretion of the physician to eliminate the possibility of diseases that exhibit symptoms similar to ALS. These diagnostic tests may include: thorough neurological examination, thyroid and parathyroid hormone levels, electomyography (EMG- recording technique that detects the muscles' electrical activity, nerve conduction velocity (NCV), blood and urine samples such as high resolution serum protein electrophoresis, 24 hour urine collection for heavy metals, spinal tap, brain or spinal cord imaging usually by magnetic resonance imaging (MRI), myelogram of cervical spine, muscle and/or nerve biopsy (Gubbay, Kahana et al. 1985; Association 2007).

Results of the NCV test may identify a patient as having a myopathy or a form of peripheral neuropathy instead of ALS. A muscle biopsy may be done if a myopathy is suspected. When a diagnosis of ALS is made, it is best to obtain a second opinion from an ALS specialist due to the similarities among ALS and a variety of other diseases. Most of the other diseases are treatable which is why it is so important to confirm an ALS diagnosis.

1.6 ETIOLOGY/CAUSE

As mentioned previously, genetics have been shown to be associated with SALS and FALS. Other hypotheses currently being investigated include: free radicals, excess glutamate, buildup of

neurofilaments, defects in mitochondria, apoptosis, and immune system abnormalities, adhesion molecule abnormalities, viruses and other infectious agents, toxins, electrical injuries, and genes.

1.7 TREATMENT

There is not a cure for ALS. There is, however, currently one FDA-approved medication to slow the progression of ALS. Riluzole (Rilutek) works by interfering with excess glutamate (Miller 2005). This treatment can extend the lifetime of a patient with ALS an additional 2-3 months. In the case of excess toxic waste products in the cell, vitamin E and other antioxidants may help to lessen the accumulation. The anti-inflammatory drugs, minocycline and TCH 346, have undergone clinical trials as potential medications to block apoptosis of motor neurons, however, the drugs were found to be unsuccessful. Currently, researchers are studying IV ceftriaxone which increases glutamate transporter activity, in hopes of finding a new treatment for ALS.

In 2008, a study in Houston, Texas, discovered mice with ALS that received a bone marrow transplant began producing T-cells thereby slowing disease progression (Alliance 2009). T-cells protect the cells in the spinal cord responsible for muscle movement due to their contact with astroglia and microglia (Alliance 2009). A protective gene (KIFAP3) was also identified that will extend survival of MND patients by 14 months, or over a 50% increase in lifetime. The study included 2,359 MND patients and 2,814 healthy volunteers from six countries to investigate 300,000 genetic variants that had been narrowed to include only those involved in MND (Alliance 2009). Two beneficial variants of KIFAP3 were found to be significant for this improvement. The role of KIFAP3 variants in this process is not fully understood although it is known to be involved in many cellular processes including transport of vital molecules in the

neurons (Alliance 2009). This finding is important because it highlights the possibility of a new area of ALS treatment which aims to improve survival.

Many of the symptoms of ALS are treatable through the assistance of a multidisciplinary team of experts including a neurologist, nurse, physical therapist, occupational therapist, social worker, speech therapist, nutritionist, patient advocacy representative and a psychologist (Miller 2005). These experts are usually available at a large ALS center.

1.8 PREVENTION

In 2005, Ascherio et al. conducted a prospective study to assess whether taking vitamin E and C on a regular basis had an effect on developing ALS among 957,740 participants 30 years of age or older in the American Cancer Society's Cancer Prevention Study II (Ascherio 2005). Regular supplementation with vitamin E resulted in lower risk of dying from ALS (RR=0.99, 95% CI: 0.69, 1.41) for occasional users, RR=0.59 (95% CI: 0.36, 0.96) for those using for less than 10 years, and RR=0.38 (95% CI: 0.16, 0.92) among regular users for 10 years or more compared to nonusers of vitamin E (p for trend = 0.004) (Ascherio 2005). Relative risks were adjusted for by smoking status and age. Vitamin E is an important cellular antioxidant involved in the reduction of membrane lipid peroxidation. Supplementation with vitamin C and multivitamins did not reduce the risk of developing ALS.

2.0 REVIEW OF LITERATURE CONCERNING ENVIRONMENTAL, OCCUPATIONAL, AND PERSONAL RISK FACTORS FOR SALS

2.1 FINDINGS OF STUDIES ON ENVIRONMENTAL OR OCCUPATIONAL EXPOSURES INCLUDING HEAVY METALS, CHEMICALS AND OTHER EXPOSURES AND ALS

2.1.1 Heavy Metals

Mercury and lead toxicity are known to produce neurological effects (Noonan 2002). Neuropathy, encephalopathy, and a syndrome similar to MND have been linked with heavy lead exposure (Noonan 2002). Because of these associations, heavy metals and lead are suspected in the etiology of MND. Chromium and hexavalent chromium have not be studied as potential risk factors for MND although the metals were found in air emission data to likely be an environmental exposure (Schulte, Burnett et al. 1996). Additionally, there is no evidence in humans or animal studies that chromium has any sort of neurological or musculoskeletal effects (Schulte, Burnett et al. 1996). It may be an interesting metal to investigate in future studies.

Trace metals including selenium, magnesium, calcium, and aluminum have been studied with regards to ALS development. A case-control study by Nelson et al. in 2000 did not find calcium, lead, copper, or mercury dietary intake to be associated with ALS risk (Nelson, Matkin et al.

2000). ALS incident cases (N=161) and 321 age and sex-matched population controls were included in the study (Nelson, Matkin et al. 2000). Aluminum was suspected to play a role in the Western Pacific variant of the disease, but has not been linked to sporadic ALS (Noonan 2002). In 2000, a case-control study of 107 cases and 262 community controls carried out by Longnecker et al. reported that dietary magnesium was protective for ALS (Longnecker, Kamel et al. 2000). Selenium levels were measured in four ALS patients in an area containing high levels of environmental selenium in South Dakota in (Noonan 2002). Investigators Schwarz, Kurland, and Kurtzke reported that increased selenium was found in one of the four ALS cases (Schwarz 1977; Noonan 2002). In the past, studies have not found any relationship between areas of high selenium environmental content in the U.S. with ALS death rates (Noonan 2002). An Italian 1996 Cohort study conducted by Vinceti et al. in examined ALS incidence among 5,182 persons exposed to high selenium levels in their water (Vinceti, Guidetti et al. 1996). The Standardized Incidence Ratio (SIR) was 4.22 (95% CI: 1.15, 10.80) for the four cases of ALS that developed over the 9-year study period (Vinceti, Guidetti et al. 1996). Only one case of ALS was actually expected for this population and during the time period specified. Other studies have produced inconsistent results with regards to selenium exposure and risk of ALS (Noonan 2002). These studies utilized blood and tissue samples.

In the 1700's, Van Sweiten wrote of a patient who had been exposed to lead and the associated muscle weakness and wasting away experienced (Campbell, Williams et al. 1970). This is thought to be the first written account of lead exposure and MND. Many studies have been conducted since that time. Exposure to lead has been associated with an increased risk of ALS by a number of studies (Felmus, Patten et al. 1976; Conradi, Ronnevi et al. 1978; Armon, Kurland et al. 1991; Chancellor, Slattery et al. 1993). The potential association of ALS and

heavy metals has also been studied (Currier and Haerer 1968; Conradi, Ronnevi et al. 1982; Gresham, Molgaard et al. 1986; Durlach, Bac et al. 1997; McGuire, Longstreth et al. 1997; Bar-Sela, Reingold et al. 2001). Campbell et al. conducted a case-control study in 1970 of 74 MND cases and 74 age and sex-matched controls to assess bone lead exposure and risk of MND (Campbell, Williams et al. 1970). Controls were selected from consecutive admissions to a general medical ward. A significant difference was not found for slight lead exposure and MND (Campbell, Williams et al. 1970). However, there was a significant different between cases and controls for severe lead exposure and MND (OR=3.1, $p<0.05$) (Campbell, Williams et al. 1970). Fifteen percent of cases had experienced severe lead exposure compared to five percent of controls (Campbell, Williams et al. 1970).

A case-control study conducted by Conradi et al. in 1978 discovered higher mean blood lead levels in 16 ALS cases compared to 18 controls (0.52 ± 0.22 μg/100 ml and 0.37 ± 0.13 μg/100 ml, respectively, $p<0.05$) (Conradi, Ronnevi et al. 1978). Controls had neurological diseases that were non-degenerative and were matched to cases by age. In 1986, a pilot case-control study conducted by Roelofs-Iverson et al. of 105 cases and 177 controls reported a significant relationship between heavy metal exposure in cases compared to controls (OR=5.3, $p<0.01$) (Roelofs-Iverson, Mulder et al. 1984; Nelson 1995). Cases included referral patients seen at the Mayo Clinic. The association of occupational heavy metal exposure (lead, mercury, metal) and ALS was assessed in a case-control study conducted by Gresham et al. in 1986 (Gresham, Molgaard et al. 1986). Sixty-six ALS cases were compared to 66 age and sex-matched controls, and no relationship was found heavy metal exposure and risk of ALS (Gresham, Molgaard et al. 1986). Acquaintance controls were used in Gresham et al.'s study. Deapen and Henderson carried out a case-control study of suspected risk factors for ALS in 1986 and only found a

significant association in cases compared to controls in plastics manufacturing (OR=3.7, 95% CI: 1.0, 20.5) (Deapen and Henderson 1986). Details about the various occupational exposures were not known. The 518 cases and 518 controls had similar exposures to other toxic substances (Deapen and Henderson 1986).

In 1991, Armon et al. carried out a case-control study among 47 ALS cases and 47 matched patient controls, and found that ALS was more common among men employed in welding or soldering jobs ($p<0.01$) and in men who worked at blue-collar jobs ($p=0.10$), compared to men without ALS (Armon, Kurland et al. 1991). A total of 74 ALS cases and 201 controls participated in the study; however, occupational and recreational data was only able to be analyzed from 47 cases and 47 controls. No women were included in the analysis due to insufficient data. Controls were matched by age and sex, and were patients without neurodegenerative disease, who were not involved in medicolegal evaluation or disability, and had a normal EMG or one that showed an isolated mononeuropathy (Armon, Kurland et al. 1991). Another case-control study was conducted in 1991 by Armon et al. of 45 ALS cases and 90 controls, and found that men exposed to $\geq$ 200 lifetime cumulative hours of lead exposure were at an elevated risk for ALS (OR=5.5, 95% CI: 1.4, 2.1, $p<0.01$) (Armon, Kurland et al. 1991). Controls were matched to cases by year of birth, sex, period of observation, and residence.

Chancellor et al. carried out a case-control study of 103 MND patients and 103 age and sex-matched community controls in 1993 to investigate risk factors for MND (Chancellor, Slattery et al. 1993). An elevated risk for MND was reported among those working in lead-exposed occupations for more than 12 months (OR=5.7, 95% CI: 1.6, 30.0, $p<0.01$) (Chancellor, Slattery et al. 1993). Eighteen percent of cases had been exposed to lead compared to only five percent

of controls (Chancellor, Slattery et al. 1993). In 2002, Kamel et al. conducted a case-control study and reported risk of ALS was elevated nearly twice the normal risk among 109 ALS cases with occupational exposure to lead compared to 256 age, sex, and region of residence frequency-matched population controls (OR=1.9, 95% CI: 1.1, 3.1) (Kamel, Umbach et al. 2002). Controls were selected by random-digit dialing. Occupational lead exposure was obtained from participants' self-report. Lifetime levels of lead exposure were related to a trend in ALS incidence (Kamel, Umbach et al. 2002). Lead levels were measured from blood and bone (tibia and patella), and blood lead levels were found to be significantly higher among cases than controls (Kamel, Umbach et al. 2002). Levels of lead in the bone were also elevated, but not as substantially so as in the blood samples (Kamel, Umbach et al. 2002). In a case-control study by Qureshi et al. in 2006, lead (p=0.02) and pesticide (p=0.03) exposure were found to be significantly associated with risk of ALS (Qureshi, Hayden et al. 2006). The researchers did their best to age-match the 95 ALS cases and 106 community controls. Cases with a shorter duration between symptoms and diagnosis as well as having a bulbar onset, experienced an accelerated progression of disease (Qureshi, Hayden et al. 2006).

Studies by Felmus et al., Armon et al., Chancellor et al., and Kamel et al. reported a significant association of exposure to lead, mercury or other heavy metals and elevated ALS or MND risk (ORs ranged from 2.0 to 6.0) (Armon 2004). The study by Felmus et al. was a case-control study of 25 ALS patients and 50 controls (25 hospitalized patients and 25 healthy people) carried out to determine factors related to the development of ALS (Felmus, Patten et al. 1976). An association of heavy mercury exposure and ALS was found among cases compared to controls (OR=6.0, p<0.05) (Felmus, Patten et al. 1976). Twenty percent of cases were exposed to heavy mercury compared to only four percent of controls (Felmus, Patten et al. 1976). Heavy

lead exposure was not associated with ALS (Felmus, Patten et al. 1976). The studies conducted by Felmus et al. and Kamel et al. may have overmatched on the exposure of interest (lead) as both studies utilized co-workers, friends, relatives, or spouses as controls (Armon 2004). These are individuals who may have also been exposed to the exposure.

More ALS patients than controls reported having been exposed to welding in a case-control study conducted by Gunnarsson et al. in 1992 (Mantel Haenszell OR=3.7, 95% CI: 1.1, 1.3) (Gunnarsson, Bodin et al. 1992). Gunnarsson et al.'s study included 92 MND cases and 372 controls of similar ages. Controls were randomly chosen from the national population register of Sweden. A study carried out in 1996 by Strickland et al. of 25 ALS cases and 50 controls also reported more contact with welding than did controls (Strickland, Smith et al. 1996). Controls were matched to cases by age and gender. Half of the controls were patients with other neuromuscular diseases, while the other half consisted of community controls selected using a random-digit dialing technique. Schulte et al. conducted a comparative study in 1996 to investigate the relation of certain occupations and neurodegenerative diseases (Schulte, Burnett et al. 1996). Between 1982 and 1991, death certificates were obtained for 27 states in the National Occupational Mortality Surveillance System to compare proportionate mortality ratios for presenile dementia, Alzheimer's disease, Parkinson's disease, and MND based upon occupation (Schulte, Burnett et al. 1996). There were 9,435 deaths from MND that were included in the study although none were significantly associated with metal working or metal casting (Schulte, Burnett et al. 1996). In 2000, Mitchell conducted a literature review of the connection between toxins and environmental exposures with ALS (Mitchell 2000). Mitchell concluded that workers in welding or soldering occupations may be exposed to lead vapor by inhalation (Mitchell 2000).

2.1.2 Agricultural Chemicals

Occupations involving agricultural chemicals have also been studied as a possible risk factor for ALS. ALS was reported to be more common among farmers and shepherds, males, and among persons 50-70 years of age as compared to the general population of Italy in a 1983 case-series of 182 ALS patients carried out by Giagheddu et al. (Giagheddu, Puggioni et al. 1983). The mean annual incidence of ALS was 0.51 per 100,000 persons and the prevalence rate was 3.65 per 100,000 (Giagheddu, Puggioni et al. 1983). The study included patients from 1957 to 1980. A case-control study of 1,961 cases and a random sample of 2,245 age-stratified population controls was carried out by Gunnarsson et al. in Sweden from 1970-1983 investigated occupations and risk of ALS (Gunnarsson, Lindberg et al. 1991). Men who were farm workers in one Swedish county were found to be at higher risk for developing ALS compared to controls (OR=1.7, CI not provided) (Gunnarsson, Lindberg et al. 1991). More male cases than male controls were office workers (OR=1.8) (Gunnarsson, Lindberg et al. 1991). A cluster of 25 ALS male cases in agricultural work was reported in one Swedish county (OR=3.4) (Gunnarsson, Lindberg et al. 1991). More female cases than female controls worked as medical service workers (OR=1.7) (Gunnarsson, Lindberg et al. 1991).

The previously mentioned case-control study conducted by Deapen et al. in 1986 also investigated pesticide exposure and risk of ALS although a statistically significant association was not found (Deapen and Henderson 1986). A case-control study of 72 MND cases in Italy was carried out by Granieri et al. in 1988 to investigate risk factors for MND (Granieri, Carreras et al. 1988). The authors reported MND occurred more often among persons living in rural areas and employed in agricultural work (OR=1.8, $p<0.05$) (Granieri, Carreras et al. 1988). It was also mentioned that persons in lower social classes experienced MND more, including those working

as unskilled and heavy laborers (Granieri, Carreras et al. 1988). Exposure to pesticides was not associated with MND (Granieri, Carreras et al. 1988). Savettieri et al. carried out a case-control study in 1991 of 46 cases and 97 closely matched healthy controls (Savettieri, Salemi et al. 1991). No relationship was found between solvents and pesticides and ALS risk (Savettieri, Salemi et al. 1991).

A case-control study conducted by Armon et al. in 1991, of 74 ALS cases and 201 age and sex-matched controls found no association between more years lived in a rural community and ALS (Armon, Kurland et al. 1991). Controls were matched to cases by ALS risk factors. Four different types of controls were used. U.S. males exposed to agricultural chemicals were found to have a higher risk of ALS compared to the general population in a case-control study in 1997 (OR=2.4, 95% CI: 1.2, 4.8) (Mitchell 2000). In 1992, a case-control study of risk factors for MND was carried out by Gunnarsson et al. in nine Swedish counties (Gunnarsson, Bodin et al. 1992). The 92 cases and 372 randomly selected population controls who participated were between the ages of 45 and 79 (Gunnarsson, Bodin et al. 1992). Male cases were more likely to work in electricity related jobs compared to controls although this finding was borderline significant (Mantel-Haenszel OR=6.7, 95% CI: 1.0, 32.1) (Gunnarsson, Bodin et al. 1992). Male cases were more often exposed to impregnating agents, however, this was also not significant, (MHOR=3.5, 95% CI: 0.9, 13.1) (Gunnarsson, Bodin et al. 1992). More male cases than controls were exposed to welding (MHOR=3.7, 95% CI: 1.1, 13.0) (Gunnarsson, Bodin et al. 1992). Risk of developing MND was influenced by family history of thyroid disease or neurodegenerative disease and was borderline significant (OR=2.1, 95% CI: 1.0, 4.3) (Gunnarsson, Bodin et al. 1992). Male cases with family history of thyroid disease or neurodegenerative disease who were exposed to solvents were found to be at greatest risk of

developing MND (OR=15.6, 95% CI: 2.8, 87.0) (Gunnarsson, Bodin et al. 1992). Gunnarsson et al. also conducted a case-control study of 168 ALS cases (107 males, 61 females) and population controls in 1996 to evaluate a cluster of MND in one Swedish county (Gunnarsson, Lygner et al. 1996). Cases with disease onset between 1961 and 1990 were included in the study. ALS male cases had greater exposure to farm work compared to the remainder of the population in 5-year intervals (Knox test disjoint procedure, p=0.02) (Gunnarsson, Lygner et al. 1996). Seventy males had ALS between 1973 and 1984 although the annual incidence was 4 per 100,000 person-years (Gunnarsson, Lygner et al. 1996). ALS was significantly higher than reported in a nearby county in spite of taking multiple comparisons into account (Gunnarsson, Lygner et al. 1996).

2.1.3 Occupational Exposures

In 1993, Chancellor et al. carried out a case-control study to assess risk factors for MND (Chancellor, Slattery et al. 1993). This study was previously described. Results of the study found an association between MND and occupational exposure to chemicals or solvents (OR=3.3, 95% CI: 1.3, 10.0) (Chancellor, Slattery et al. 1993). No relationship was found for MND and pesticides, minerals, or ores (Chancellor, Slattery et al. 1993). An assessment of agricultural chemicals, metals, and solvents and ALS was carried out in a case-control study of 174 ALS cases and 348 age and sex-matched community controls in 1997 by McGuire et al. (McGuire, Longstreth et al. 1997). Controls were identified through random-digit dialing or Medicare enrollment files. Risk of ALS was elevated two-fold in those ever exposed to agricultural chemicals after adjusting for education and age (OR=2.0, 95% CI: 1.1, 3.5) (McGuire, Longstreth et al. 1997). The association of ALS and agricultural chemicals was seen in men but not in women demonstrating that sex was a confounder in the model (OR=2.4, 95%

CI: 1.2, 4.8) (McGuire, Longstreth et al. 1997). These results were based on the report of a panel of blinded industrial hygienists. No relationship was found between ALS and solvents and metal exposure (McGuire, Longstreth et al. 1997). The risk of ALS and exposure to agricultural chemicals was also assessed in the review conducted by Mitchell in 2000 (Mitchell 2000). Rosati et al.'s study in Sardinia, Italy, found an increased incidence of ALS among agricultural workers (5.28 cases per 100,000), as well as among shepherds and farmers compared to the general population (Rosati, Pinna et al. 1977).

Researchers have examined the potential relationship between electrical work and/or electrical shocks and ALS. Deapen and Henderson carried out a case-control study of 518 ALS cases and 518 controls in 1986 and found that more cases than controls reported working in occupations with risk of electrical exposure (OR=3.8, 95% CI: 1.4, 13.0) (Deapen and Henderson 1986). Electrical shocks resulting in unconsciousness occurred more often among cases than controls although this result was borderline statistically significant (OR=2.8, 95% CI: 1.0, 9.9) (Deapen and Henderson 1986). Cases also reported they were at greater risk of electrical shock resulting in unconsciousness due to their occupations although this increase was not significant (OR=2.8, 95% CI: 1.0, 9.9) (Deapen and Henderson 1986). Mitchell's review found more ALS patients had experienced electrical shock(s) compared to age and sex-matched controls (OR=3.50, 95% CI: 1.18, 10.34) (Mitchell 2000).

The association of ALS and exposures to leather, rubber, solvents and chemicals has also been investigated. An increased risk of ALS was reported among workers involved with animal hides and carcasses, and leather work (Mitchell 2000). An association between rubber work and ALS was reported by Mitchell in 2000 although ALS was not found to be associated with animal carcasses or hide or leather work (Mitchell 2000). A confounding effect may be taking place in

the studies, suggesting increased risk of ALS due to leather work. Chemicals and solvents used in the workplace should be taken into consideration.

Since the 1970's, many research studies have attempted to explain potential causes for ALS. In summary, some associations have been established such as though of welding/soldering and lead and ALS, although one study did not find an association between lead exposure and ALS. Other areas of concern include: electrical exposure or shocks; certain occupations including farming, shepherds, and others; exposure to chemicals and pesticides; heavy metals; leather or animal hides/carcasses; and heritability to thyroid or neurodegenerative diseases have produced inconsistent results. Consequently, more research must be done in these areas to determine whether an association truly does exist between the suspected exposures and risk of ALS.

Hyser et al. reported an occupational cluster of ALS in Ohio in 1987 among three teachers who had taught in the same classroom for 2-5 years (Hyser, Kissel et al. 1987). This case-series found that ALS developed in each of the teachers in an 18-month time period (Hyser, Kissel et al. 1987) Only 0.7 deaths were expected for the school over this time period, and the probability of three ALS deaths occurring was $p<0.0001$ (Hyser, Kissel et al. 1987). For all the schools in Ohio, the probability of three or more teachers dying from ALS was $p<0.05$ (Hyser, Kissel et al. 1987). The three teachers had nothing in common other than working at the same school and in the same classroom for 2-5 years.

The association between occupational exposures in females and ALS has also been studied. In 2007, Sutedja et al. carried out a case-control study of 364 ALS patients and 392 age and sex-matched acquaintance controls to evaluate lifetime occupation, education and smoking in relation to ALS (Sutedja, Veldink et al. 2007). Controls could not be a relative, spouse, or partner. The authors found women who worked in the fields of crafts and related trades workers

were at a higher risk of ALS (OR = 8.4; 95% CI = 1.0 to 70.1; p = 0.05) (Sutedja, Veldink et al. 2007). Other female-held occupations were associated with increased risk of ALS although they were not significant (Sutedja, Veldink et al. 2007). A cohort study of 937 ALS cases (507 men, 430 women) carried out by Weisskopf et al. in 2005, found an elevated risk of ALS among female machine assemblers (Rate ratio=2.81, 95% CI: 1.05, 7.53, *p*=0.04) (Weisskopf, McCullough et al. 2005).

2.2 STUDIES OF CIGARETTE SMOKING, TOBACCO USE, ALCOHOL CONSUMPTION, AND OTHER CHARACTERISTICS OF ALS

A number of studies have explored the potential association between smoking, alcohol, and/or drug use and ALS. The number of years one has smoked, the quantity, and current smoking are relative to the risk of ALS. Five case-control studies and three prospective cohort studies were carried out to investigate smoking and ALS. Weisskopf et al. carried out a cohort study of 621 patients (291 women, 330 men) who died from ALS in the Cancer Prevention Study II cohort in 2004 to investigate smoking and ALS mortality (Weisskopf, McCullough et al. 2004). The authors found significantly elevated ALS mortality rates for women who currently smoked compared to those who did not currently smoke ($p<0.0003$) (Weisskopf, McCullough et al. 2004). Women who currently smoked had an increased risk for ALS mortality (RR=1.67, 95% CI: 1.24, 2.24; $p=0.002$) compared to the risk for ALS mortality in men who currently smoked (RR=0.69, 95% CI: 0.49, 0.99, $p=0.04$) (Weisskopf, McCullough et al. 2004). A cohort study conducted by Fang et al. in 2006, examined smoking and snuff dipping in the Swedish Construction Workers Cohort of over 280,000 male workers (Fang, Bellocco et al. 2006). The

Swedish Inpatient Register was used to identify incident ALS cases. Participants were followed for a mean duration of 19.6 years from study entry. Smoking and snuff dipping were not significantly associated with an excess risk of ALS, (RR=0.8, 95% CI: 0.6, 1.1) and (RR=0.6, 95% CI: 0.3, 1.5), respectively (Fang, Bellocco et al. 2006). Stratifying data by smoking status or type of smoking did not have any additional effects on the previously reported risks (Fang, Bellocco et al. 2006).

Another case-control study carried out in 1999 by Kamel et al. assessed smoking and ALS among 109 cases and 256 population controls. Controls were identified by random telephone screening, while cases were included from two referral centers. A borderline significant relationship was found between ever having smoked and ALS risk (OR=1.7, 95% CI: 1.0-2.8) (Kamel, Umbach et al. 1999). The model was adjusted for education, age, sex, and region. Although no dose-response trends were present, ALS cases had smoked more cigarettes and for a longer duration compared to controls (Kamel, Umbach et al. 1999). Cases drank slightly more alcohol than controls; however, this difference was not significant and smoking was likely the confounder (Kamel, Umbach et al. 1999). Nelson et al. conducted a case-control study of 161 cases and 321 matched population controls in 2004 to evaluate smoking and alcohol use (Nelson, McGuire et al. 2000). Controls were selected by random-digit dialing and Medicare enrollment files. Results were adjusted for gender, age, education, and respondent type. Alcohol was not linked to ALS risk, but smoking was found to be related. The risk was highest for current smokers (OR=3.15, 95% CI: 1.9, 6.4) (Nelson, McGuire et al. 2000). Having ever smoked cigarettes was associated with a two-fold increase in risk (OR=2.0, 95% CI:1.3, 3.2) and history of smoking a non-significant increased risk (OR=1.5, 95% CI: 0.9, 2.4) (Nelson, McGuire et al. 2000). Number of pack-years and duration of smoking were both significantly associated with a

dose-response trend in incidence of ALS (p for trend=0.001, for each) (Nelson, McGuire et al. 2000).

A case-control study of 219 cases and 254 controls conducted by Veldink et al. in 2005, found that current or ever consumption of alcohol was protective for ALS (OR=0.6, 95% CI: 0.3, 0.9, p=0.04). In addition, current smoking was related to elevated risk of ALS (OR=1.8, 95% CI: 1.0, 3.0, p=0.03) (Veldink, Kalmijn et al. 2005). In Veldink's study, case-identified population controls were matched to cases by age and sex. In 2007, a case-control study by Sutedja of 364 ALS cases and 392 controls, that was previously described, was carried to evaluate lifetime occupation, education and smoking in relation to ALS (Sutedja, Veldink et al. 2007). In univariate analyses, risk of ALS was highest among women whose occupation was classified as crafts or related trade workers as defined by the International Classification of Occupations (OR=8.4, 95% CI: 1.0, 70.1, p=0.05) (Sutedja, Veldink et al. 2007). Those with elementary education levels were at an increased risk for ALS (OR=2.2, 95% CI: 1.2, 3.8, p<0.001) (Sutedja, Veldink et al. 2007). Current smoking was associated with elevated risk for ALS, as well (OR=1.7, 95% CI: 1.1, 2.6, p=0.01) (Sutedja, Veldink et al. 2007). Current smoking was also associated with elevated risk for ALS in the multivariate analysis (OR=1.6, 95% CI: 1.0, 2.5, p=0.04) (Sutedja, Veldink et al. 2007).

A cohort study carried out by Qureshi et al. in 2006, followed 95 ALS patients for one year to evaluate factors that modify susceptibility and rate of progression in ALS (Qureshi, Hayden et al. 2006). Qureshi et al. also conducted a case-control study with the same study population and 106 healthy controls. Cases and controls had similar demographic characteristics at study entry. Smoking, a history of physical trauma, a history of clinical disorders, and physical activity were not linked with causation or disease progression of ALS (Qureshi, Hayden et al. 2006).

In conclusion, the data on smoking, tobacco, and alcohol use are conflicting. Cigarette smoking has been evaluated in a number of studies although more research needs completed to better assess whether a potential relationship exists between smoking and ALS. In addition, alcohol and tobacco use should also be further investigated since few studies have been carried out in these areas regarding ALS research.

2.3 STUDIES OF TRAUMA AND INJURY IN ALS

Some researchers believe that risk of developing ALS may be related to a history of trauma or injury. The risk of ALS associated with skeletal trauma is one of the most controversial. A review article by Kurland et al. in 1992 reported finding no association between mechanical trauma and ALS based on a review of the literature (Kurland, Radhakrishnan et al. 1992). The authors stressed the importance of examining trauma and the risk of ALS in a prospective design. One retrospective cohort and eight case-control studies assessed the relationship between trauma or injury and ALS (Chen, Richard et al. 2007). The majority of studies did not find a relationship between ALS and head trauma or injury. A possible explanation for the inconsistency of study results is the unclear definition of head injury or trauma, or when specified, a variation of the definition among studies. Additionally, the use of convenient controls, small sample sizes, lack of adjustment for potential confounders, and inadequate exposure assessment all contributed to the contradictory results (Kurland, Radhakrishnan et al. 1992; Chen, Richard et al. 2007). As a result, bias, confounding, and low power were also implicated.

In 1980, a case-control study conducted by Kurtzke and Beebe of 504 World War II veterans who had died of ALS and 504 military controls reported no association of head injury and ALS (OR= 1.0, 95% CI: 0.1, 7.1) (Kurtzke and Beebe 1980). Head injury was not explicitly defined in this study. Cases and controls were matched by date of entry into military service, year of birth, and branch of service. In the 1990's, a population-based case-control study conducted by Cruz et al. in western Washington state investigated the role of physical trauma including electrical shocks, fractures, and surgeries in 174 ALS cases and 348 controls identified by random digit dialing or Medicare lists, and found no significant association (Cruz, Nelson et al. 1999). Two controls were matched by age and gender to each case. A case-control study carried out by Gallagher and Sanders in 1987 found the presence of head or neck injury was not significantly associated (OR=1.7, 95%CI: 0.8, 3.4) (Gallagher and Sanders 1987). The study included 135 ALS cases who had developed ALS before the age of 45, and 85 multiple sclerosis controls. Gresham et al. carried out a case-control study of 66 cases (33 male, 33 female) and 66 age and sex-matched controls in 1987 exploring ALS and head injury, not specifically defined (Gresham, Molgaard et al. 1987). No results were concluded as the numbers were not consistent throughout the text (Gresham, Molgaard et al. 1987). Controls were neighbors or friends of ALS cases, but not former co-workers. Granieri et al.'s study which was previously discussed, also found MND risk was increased in cases compared to controls with had a history of trauma (Granieri, Carreras et al. 1988).

In 1991, Williams et al. conducted a retrospective cohort study of 821 head trauma victims who were thought to have brain injuries, and found no association between head trauma and ALS (SMR=1.05, 95% CI: 0.027, 5.85) (Williams, Annegers et al. 1991).

A recent case-control study of 110 ALS cases and 256 population controls conducted by Chen et al. in 2007 reported no association between history of a head injury and ALS (OR=1.4, 95% CI: 0.8, 2.6) (Chen, Richard et al. 2007). Controls were frequency-matched to cases by area code, age, and gender and selected from random telephone screening. Risk of ALS was found to be significantly higher for persons with more than one head injury compared to those without a head injury (OR=3.1, 95% CI: 1.2, 8.1) (Chen, Richard et al. 2007). Risk of ALS was also increased for those who had a head injury within the last 10 years compared to those not experiencing a head injury (OR=3.2, 95% CI: 1.0, 10.2) (Chen, Richard et al. 2007). An 11-fold increase in risk of ALS was reported for persons who experienced multiple head injuries, the most recent of which had occurred in the past 10 years in a post-hoc analysis of a small sample (Chen, Richard et al. 2007). Compared to cases without head injuries, cases with head injuries experienced an earlier age of ALS diagnosis (54 years compared to 59.5 years; p=0.05), and more often had a bulbar onset (33.3% compared to 22.4%; p=0.3) (Chen, Richard et al. 2007).

However, conflicting results have also been reported. Two case-control studies conducted in 1981 by Kondo and Tsubaki established a significant relationship between ALS and head injury, which was not specifically defined (OR=5.6, 95% CI: 2.5, 12.6) (Kondo and Tsubaki 1981). The first study consisted of 712 ALS cases and the second study involved 158 ALS cases (Kondo and Tsubaki 1981). The results concluded that mechanical injuries were a risk factor for motor neuron disease but were not the cause (Kondo and Tsubaki 1981). "Smoking, drinking, residence, home space, drinking water, animals, experience as a war prisoner, stay on Guam, parental consanguinity, measles, polio, mumps, tuberculosis, rheumatism, prosthesis of the total teeth, shell splinters retained in the body, occupational exposures to radiations, chemicals, or

gases, atomic bombings, electric injuries, surgical operations, and occupations" were not associated with risk of ALS (Kondo and Tsubaki 1981).

A borderline significant association was described in a case-control study by Deapen and Henderson in 1986 for ALS and unconsciousness due to external (nonelectrical) trauma (OR=1.6, 95%CI: 1.0, 2.4) (Deapen and Henderson 1986). The study included 518 ALS cases and 518 controls. Strickland et al. A carried out a case-control study in 1996 and found more severe head, neck, and back injuries among cases than controls (OR=5.3, 95% CI: 1.7, 17.0) (Strickland, Smith et al. 1996). Two controls, one from the community and one from the muscle disease clinic in which cases were drawn, were matched to each ALS patient. Controls from the muscle disease clinic had diseases other than ALS. In addition, ALS patients reporting previous injuries were younger than controls at the time of the injury (Strickland, Smith et al. 1996). As for bone fractures in cases and controls, there was no significant difference among the two groups other than a non-significant age difference which suggests bones may have been broken in cases at a younger age than controls (Strickland, Smith et al. 1996). Chen et al. conducted a meta-analysis in 2007 of eight published studies on ALS and head injury between 1980 and 2007 (Chen, Richard et al. 2007). Results of the meta-analysis found persons with past head injuries had a somewhat increased risk of ALS (OR=1.7, 95%CI: 1.2, 2.2) compared to persons without head injury (Chen, Richard et al. 2007). Gawel et al conducted a case-control study in 1983 and found more ALS patients had experienced back injuries compared to controls (X^2=12.88, p<0.01) (Gawel, Zaiwalla et al. 1983). Controls more often experienced head injuries and fractures than ALS patients (Gawel, Zaiwalla et al. 1983).

As stated previously, studies of the relationship between skeletal trauma and risk of ALS have been very inconsistent. Many of the studies have also had limitations which were

previously discussed. As a result, this area is still in need of further studies to better clarify the role played by skeletal and head traumas in relation to ALS risk.

2.4 FINDINGS OF STUDIES OF PHYSICAL ACTIVITY AND ASSOCIATED TRAUMA IN ALS

It has been hypothesized that persons who engage in competitive sports and who are athletic are at an increased risk of developing ALS. A case-control study conducted by Veldink et al. in 2005 examined physical activity at both work and leisure and the risk of developing sporadic ALS among 219 cases and 254 controls (Veldink, Kalmijn et al. 2005). Case-identified population controls were matched to cases by age and sex. Also assessed were duration of ALS and the onset age in relation to physical activity. A systematic review of the literature was conducted as well as three cumulative measures of physical activity for the study, namely, early physical activity (up until 25 years of age), late physical activity (10 years before disease onset), and total physical activity (up until one year prior to disease onset) (Veldink, Kalmijn et al. 2005). As demonstrated through the systematic review and the current study, Veldink et al. found no relationship between ALS and physical activity (Veldink, Kalmijn et al. 2005).

Studies by Belli and Chio reported a significant association between ALS and Italian soccer players who had a bulbar onset and a previous head injury (Belli and Vanacore 2005; Chio, Benzi et al. 2005). In 2005, Belli et al. reported that ALS mortality was 12 times higher than expected among 24,000 Italian professional soccer players compared to the expected ALS rate for the population (Belli and Vanacore 2005). A confirmation study conducted by Chio et al. in 2005 evaluating Italian soccer players' incidence of ALS between 1970 and 2001 found

incidence for ALS in five soccer players was increased by 6.5-fold compared to the general population (Chio, Benzi et al. 2005). Furthermore, Italian soccer players diagnosed with ALS were more likely to have bulbar involvement and early onset (Chio, Benzi et al. 2005). Another study carried out in 2007 by Wicks et al. reported a cluster of concurrent ALS among three amateur soccer player friends (Wicks, Ganesalingham et al. 2007). The friends were also from the same area in southern England. Researchers have conjectured as to the reason for the association including the possibility of excessive physical activity, pesticides used on the field, the use of performance enhancing drugs, dietary supplements, or trauma to the head or other body parts (Chio, Benzi et al. 2005; Migliore and Coppede 2009). A case report by Abel found U.S. professional football players from the NFL (National Football League) were identified as having an 40-fold higher prevalence rate (206 per 100,000) for ALS than the general population's prevalence rate of 5 per 100,000 ($p<.001$, binomial theorem) (Abel 2007).

Vigorous physical activity and ALS have also been found not to be associated by some studies (Roelofs-Iverson, Mulder et al. 1984; Granieri, Carreras et al. 1988). When studying the relationship between traumas due to physical activity and incidence of ALS, it is important to control for physical activity to help alleviate confounding. In summary, data from studies on physical activity and associated traumas suggest that more research must be done in these areas. Physical activity in general was not shown to be associated with risk of ALS, but professional soccer and football players had significantly higher rates of the disease. Additional research should help to explain the differing results.

The association of body mass and ALS or MND has also been investigated. Scarmeas et al. conducted a case-control study of 279 cases with MND and 152 controls to assess the association of body mass and a history of athleticism with ALS (Scarmeas, Shih et al. 2002). Controls had

neurological diseases other than MND. More MND patients than controls reported that they had been varsity athletes (OR=1.70, 95% CI: 1.04, 2.76) or had been slim (OR=2.21, 95% CI: 1.40, 3.47) (Scarmeas, Shih et al. 2002). Two other studies also reported higher risk of ALS among slimmer persons (Kurtzke and Beebe 1980; Roelofs-Iverson, Mulder et al. 1984).

2.5 STUDIES OF MILITARY HISTORY RELATED TO ALS

It has been suggested that serving in the U.S. military may be linked to ALS. Horner et al. investigated ALS among Gulf War Veterans including active duty military and mobilized Reserves who served for at least one month, utilizing a case-series study design from August 1990 to July 1991 (Horner, Kamins et al. 2003). Those who served in the National Guard were also recruited to participate in the study. Eligible military personnel consisted of nearly 2.5 million, of which there were 107 ALS cases (Horner, Kamins et al. 2003). The study included ALS cases for ten years since August 1990. They found veterans who served in specific sectors of the Gulf War had elevated rates of ALS compared to the age-adjusted, 10-year average cumulative incidence rates. In particular, it was reported that all deployed personnel, deployed active duty members, and deployed Air Force and Army personnel were at an increased risk of developing ALS, respectively (RR=1.92, 95% CI: 1.29, 2.84); (RR=2.15, 95% CI: 1.38, 3.36); (RR=2.68, 95% CI: 1.24, 5.78); (RR=2.04, 95% CI: 1.10, 3.77) (Horner, Kamins et al. 2003). Deployed Navy, Marine Corps, Reserves and National Guard personnel also experienced a higher risk of ALS, although the risk was non-significant possibly due to small numbers (Horner, Kamins et al. 2003). Deployment, in general, was linked to an attributable risk of ALS of 18%

(95% CI: 4.9%, 29.4%) (Horner, Kamins et al. 2003). Self-reported deployment status rather than deployment status as determined by DMDC records provided higher RRs.

In 2003, a case-control study conducted by Haley assessed ALS cases of Gulf War veterans diagnosed before 45 years of age (young veterans) compared to age-specific U.S. population death rates between the years, 1991 and 1998 (Haley 2003). The incidence of ALS observed in the young vets rose from one case/year in 1991 to five cases/year in 1998, while the expected incidence only increased slightly from 0.93 to 1.57 cases/year (Haley 2003). This is demonstrated by the elevation in incidence over the eight year period. At baseline, the observed versus expected incidence was 4 vs. 4.25 cases (p=.06), or 0.94-fold the expected incidence (Haley 2003). Midway through at four years, the observed versus expected incidence had risen to 13 vs. 5.72 cases (p=0.006), or 2.27 times the expected incidence (Haley 2003). At the study's end and after eight years, the observed versus expected incidence was the highest at five vs. 1.57 cases (p=0.02), or a 3.19-fold elevation in the expected incidence (Haley 2003). Young vets experienced ALS incidence rates higher than the incidence rates expected for ALS with a significant trend (Poisson trend test, p=0.05) (Haley 2003). Neither a change in the U.S. population death rate nor an alteration in the onset to diagnosis interval could elucidate this rise in incidence of ALS.

A cohort study of over 500,000 U.S. men conducted by Weisskopf et al. between 1989 and 1998 investigated ALS mortality and military service. The cohort was constructed of eligible men from the American Cancer Society's Cancer Prevention Study II (CPS II). The questionnaire for women did not include questions on military service; therefore, women were unable to participate in the current study. An increased risk of ALS mortality was found for men who served in the military compared to those who didn't (relative risk (RR)=1.53, 95% CI: 1.12,

2.09, p=0.007) (Weisskopf, O'Reilly et al. 2005). The risk remained elevated in all military branches except the Marines (Weisskopf, O'Reilly et al. 2005). This may be due to the small number of Marines in the study. Every 5-year birth cohort experienced a greater risk for ALS from 1915 to 1939 (Weisskopf, O'Reilly et al. 2005).

In an editorial article published in *Neurology* in 2005, Beghi and Morrison cogitated on previous studies of ALS and military service (Beghi and Morrison 2005). They discussed the study by Weisskopf et al. in which an increase in ALS was found regardless of branch and time served, for the most part, which discredits prior findings in which ALS was thought to have developed due to specific toxins related to the Gulf War alone (Beghi and Morrison 2005). Beghi and Morrison also opine that the slightly increased risk of ALS among persons who served in the military will continue (Beghi and Morrison 2005). Armon pointed out that the elevated risk of ALS in deployed Gulf War veterans may illustrate a reduction in risk among non-deployed veterans (Armon 2004).

Schmidt is presently carrying out a case-control study known as the GENEVA study, to evaluate genes and environmental exposures in ALS vets compared to veteran controls (Schmidt, Allen et al. 2008). Thus far, 537 cases have been enrolled (85% of the targeted number) and 292 controls or 30% of the final sample required (Schmidt, Allen et al. 2008). This study will add to the literature with regards to ALS and previous military history as well as specific exposures that may be potentially associated.

In conclusion, the studies found an association between military history and risk of ALS. This was especially true for veterans of the Gulf War. When the military branches were broken down, the results were somewhat different depending on the branch. The associations should be

further explored to better determine what veterans could have specifically exposed to as any genetic susceptibility they may possess.

2.6 FINDINGS OF STUDIES OF EXCESS GLUTAMATE

A popular theory among researchers is that an excessive amount of the neurotransmitter glutamate that stimulates motor neuron cells is associated with ALS. Glutamate is an amino acid normally found in the brain and spinal cord that helps with normal growth and metabolism as well as communication between neurons. It has been discovered that in ALS, glutamate builds up in the spinal cord's motor neurons (Ludolph, Langen et al. 1992).

2.7 EXCESS OF ABNORMAL PRODUCTS OF METABOLISM

Researchers have also been interested in investigating the effects of the excess of abnormal products of metabolism.

2.8 EXCESSIVE TOXIC WASTE PRODUCTS IN THE CELL

Excessive toxic waste products in the cell became an area of interest particularly after the discovery of the SOD-1 gene mutation. Cells regularly expend energy and conduct processes during which accumulation of oxidized substances occurs also known as free radicals or reactive oxygen species.

2.9 IMMUNITY AND INFLAMMATION IN THE BRAIN AND SPINAL CORD AND ALS

The degeneration and death of motor neurons are suspected to be related to altered immune responses (Beghi, Mennini et al. 2007). Antibodies to voltage-gated calcium channels as well as anti-Fas autoantibodies found in the blood of a quarter of ALS patients are predicted to be due to autoimmunity (Beghi, Mennini et al. 2007).

Inflammation within the brain and spinal cord is another research area of interest. People with ALS are thought to have too much inflammation within motor neurons. There is some evidence to support this assumption. Some researchers think ALS development may be due, in part, to an activation and misdirection of the body's immune system (Miller 2005). In addition, neuroinflammatory elements may be involved in the development of ALS as patients with ALS have been reported to have greater levels of monocyte chemoattractant protein-1 (MCP-1), interleukin (IL)-6, and tumor necrosis factor (TCF) (Beghi, Mennini et al. 2007). Moreover, scientists have been investigating a protein present in degenerative motor neurons of patients, known as peripherin (Beghi, Mennini et al. 2007). Uncovering the role of inflammatory cytokines on peripherin expression may be important if it turns out to be associated with an overexpression of the protein (Beghi, Mennini et al. 2007).

Although scientists have been searching for any potential connections between viruses and ALS, no association has been presently found regarding this relationship. Viruses are still suspected and more research needs to be conducted on amyotrophy and the selective death of motor neurons (Beghi, Mennini et al. 2007). It has been hypothesized that infection with enteroviruses such as poliovirus are associated with an increased rate of ALS (Beghi, Mennini et al. 2007).

3.0 PESTICIDE EXPOSURE AS A RISK FACTOR FOR AMYOTROPHIC LATERAL SCLEROSIS: A META-ANALYSIS OF 11 EPIDEMIOLOGICAL STUDIES

Angela M. Malek, Ph.D.[1]

Aaron Barchowsky, Ph.D. [2]

Robert Bowser, Ph.D. [3]

Ada Youk, Ph.D. [4]

Evelyn O. Talbott, Dr.P.H, M.P.H. [1]

[1] Department of Epidemiology, University of Pittsburgh,
Graduate School of Public Health, Pittsburgh, PA 15261

[2] Department of Environmental and Occupational Health, University of Pittsburgh,
Graduate School of Public Health, Pittsburgh, PA 15261

[3] Department of Pathology, University of Pittsburgh,
College of Medicine, Pittsburgh, PA 15261

[4] Department of Biostatistics, University of Pittsburgh,
Graduate School of Public Health, Pittsburgh, PA 15261

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3.1 ABSTRACT

Background: Exposure to pesticides and agricultural chemicals has been linked to Amyotrophic Lateral Sclerosis (ALS) although findings have been inconsistent. A meta-analysis of studies published through May, 2011 was conducted to investigate the association of pesticide exposure and risk of ALS.

Methods: Eleven peer-reviewed, published studies that met criteria were included in the meta-analysis of 2,432 ALS and motor neuron disease cases. Contingent upon heterogeneity across studies, a random effects or fixed effect model was used to calculate overall and sex-specific pooled odds ratios (ORs).

Results: Evidence was found for a significant association of exposure to pesticides and ALS in cases compared to controls (OR=1.85, 95% CI: 1.27-2.71) although the chemical or class of pesticide was not specified by the majority of studies. The study specific ORs were considered heterogeneous at $p<0.10$.

Conclusion: This meta-analysis supports the association of exposure to pesticides and ALS among all cases and among male cases compared to controls. The weight of evidence links pesticide exposure to ALS; however, additional prospective studies with a target exposure group are necessary to better elucidate the relationship. The public health significance of this study is that it explores the relationship of exposure to pesticides and risk of ALS, an area of concern that is worthy of additional research. Future research should focus on more accurate exposure assessment, the use of Job Exposure Matrices (JEM), and the inclusion of biological specimens.

Key words: ALS, pesticides, occupational exposures, epidemiology, meta-analysis

3.2 INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disease (MND) in adults with an incidence of approximately 1-3 per 100,000 persons worldwide each year (Doi, Kikuchi et al. 2006; Migliore and Coppede 2009). Men have a 50% greater risk of developing ALS compared to women although the inequality seems to balance out after menopause (Kamel, Umbach et al. 2005; Migliore and Coppede 2009). ALS risk increases with age with an average age of onset of 58-63 years for sporadic ALS (Kamel, Umbach et al. 2005; Doi, Kikuchi et al. 2006). After 75 years of age, ALS incidence decreases (Migliore and Coppede 2009). ALS is characterized by progressive degeneration of both the upper and lower motor neurons resulting in muscle weakness, atrophy, impaired respiration, and ultimately death (Borasio and Miller 2001). The median survival after onset of ALS is about 2-4 years (Borasio and Miller 2001).

There are very few known risk factors for ALS identified from previous epidemiologic investigations, and those identified are very general and include male sex and age (Nelson 1995; Morahan and Pamphlett 2006). The 3.0 to 2.0 male to female ratio argues for a possible environmental or occupational exposure not experienced in a widespread manner in women. Genetic susceptibility to various environmental exposures is also suspected to be related to ALS.

Since 1950, pesticide use has risen over 50% and pesticide toxicity has increased ten-fold (Miller 2006). The largest proportion of pesticides in use are herbicides with insecticides comprising the second largest group (McKinney 2007). Three million cases of acute severe pesticide poisoning and over 200,000 deaths are reported annually and include both occupational exposures as well as general exposures (Keifer and Mahurin 1997; EPA 2011). Pesticides, many of which are related to widespread agricultural application, are considered to be potentially neurotoxic.

Previous studies have reported an association between pesticide exposure and risk of Parkinson's and Alzheimer's disease (Elbaz, Dufouil et al. 2007; Stozicka, Zilka et al. 2007; Migliore and Coppede 2009). Moreover, persistent neurological deficits and neurological diseases such as ALS and Parkinson's Disease, in which the mechanism is oxidative stress resulting from exposure to pesticides with characteristics similar to rotenone, paraquat, maneb, and dieldrin, may be linked to longer-term lower level pesticide (Kamel and Hoppin 2004; Migliore and Coppede 2009). In a few cases, herbicides have been associated with changes in neurobehavioral performance (Dobbs 2009). Neurotoxicity of pesticide exposure at moderate levels is debatable.

The main varieties of toxic pesticides include: (1) organophosphates (OPs), (2) carbamates, (3) organochlorines, (4) fungicides, and (5) fumigants. OPs, are used as insecticides, herbicides, and in chemical warfare as nerve gases due to their ability to paralyze smooth and striated muscles (Cecchine 2000). OPs inhibit the enzyme acetylcholinesterase (AChE) resulting in overstimulation of postsynaptic cholinergic receptors and an excess of acetylcholine at neuromuscular junctions and in the brain (Keifer and Mahurin 1997). The use of OPs is declining as pyrethrins, natural and supposedly non-toxic pesticides have become the most common current exposure to millions of people. Pyrethrins are produced naturally from the chrysanthemum plant and are used as insecticides, fogging products, and for some pet products. Neurotoxicity of pyrethrum (pyrethrins and pyrethroids) to humans remains somewhat controversial; however, skin allergies have been reported (EPA 2011).

Carbamates are used as insecticides and pharmaceuticals, and are the most commonly used household products. Carbamates (2,2,dimethyl-1,3-benzodioxol-4-yl methylcarbamate; 2,3-isopropylidene-dioxyphenyl methylcarbamate) also inhibit AChE although the effects are

reversible (Cecchine 2000). Depending on the duration and intensity of exposure, carbamates and OPs can cause a number of symptoms such as: fasciculations, muscle contractions, weakness, mood changes, cognitive effects, seizures, neuromuscular paralysis, respiratory failure, and even death. OPs and carbamates inhibit the enzyme acetycholinesterase (AChE) resulting in overstimulation of postsynaptic cholinergic receptors and an excess of acetylcholine at neuromuscular junctions and in the brain (Keifer and Mahurin 1997).

Organochlorines or chlorinated hydrocarbons include the banned pesticide DDT as well as dicofol, heptachlor, endosulfan, chlordane, aldrin, dieldrin, endrin, mirex, lindane, and pentachlorophenol. Some organochlorines are stored in fat tissue. A hyperexcitable state takes place in the brain following acute exposure and the endocrine system also seems to be affected by these pesticides. Symptoms of poisoning involve sensory disturbances, convulsions, and even death. In Parkinson's Disease models, oxidative stress was found to result from exposure to pesticides with characteristics similar to rotenone, paraquat, maneb, and dieldrin (Migliore and Coppede 2009). Decreased levels of SOD1 and glutathione reluctase have also occurred from OP and other pesticide exposures (Migliore and Coppede 2009).

Fungicides include: cloroneb, chlorothalonil, dicloran, hexachlorobenzane, and pentachloronitrobenzene. Fungicides generally do not cause systemic poisoning aside from organomercury poisoning as mercury is known to produce neurotoxic effects (Felmus, Patten et al. 1976; Noonan 2002). Some effects of fungicide poisoning include liver injury, corneal opacities, pyrexia, muscle wasting, and skin conditions.

Fumigants include gases, liquids, or solids, and include a large variety of products. Poisoning by some fumigants produces serious central nervous system effects as well as liver

and kidney damage, respiratory effects, progressive paralysis, convulsions, coma, shock, tremor, cardiac conditions, respiratory distress, and even death.

It is well known that acute high level pesticide exposure to OPs, carbamates, organochlorine, fungicides and fumigants affect the nervous system (Kamel and Hoppin 2004). Paraoxonase 1 (PON1) is an enzyme that hydrolyzes OPs; therefore, those with higher levels of PON1 have less toxicity as they are able to metabolize even higher doses of OPs. Single nucleotide polymorphisms (SNPs) in the PON1 gene were found to be related to sporadic ALS through the mechanism of alteration of PON function by six studies (Ticozzi, LeClerc et al.).

To date, no meta-analyses have been carried out to investigate the association between pesticide exposure and risk of ALS. This meta-analysis will focus on the broad category of occupational exposure to pesticides in order to evaluate the overall risk estimates presented in the peer-reviewed literature to date.

3.3 MATERIAL AND METHODS

3.3.1 Study identification

A systematic search of published articles in Pubmed was conducted to identify epidemiological studies of the association between exposure to pesticides and risk of ALS or MND through May, 2011. MND was included because the majority of MND cases are ALS. The database was searched for potential studies to be included in the meta-analysis using the following MeSH terms: amyotrophic lateral sclerosis or ALS or motor neuron disease or MND in combination with agrochemicals or pesticides. Studies containing gardening-related exposures were excluded

to eliminate the potential confounding effect of hobby-related exposures. The search method for studies included in the meta-analysis along with exclusion criteria is shown by the QUOROM diagram (Figure 1). The literature search identified 141 studies of which 69 were relevant to neurological disease. In addition, references from primary and review articles were manually reviewed to identify any relevant articles; thirteen studies were identified in this manner. Of the 82 relevant studies (69 from the Pubmed search and 13 from the manual review), eleven met the inclusion criteria of: (1) peer-reviewed, (2) case-control or prospective cohort studies, (3) published in the English language, and (4) provided measures of odds ratios (OR) or relative risks (RR) (e.g., unadjusted or adjusted OR) for ALS or the number of individuals (either cases and controls, or cases and person-years), and were therefore included in the analysis. Review articles, case-series, commentaries, laboratory science studies, and any non-relevant studies were excluded from the analysis.

Standardized data extraction forms were used to extract the following data from each included study: location, year, study design, source of cases/controls, diagnostic criteria, pesticide exposure source, number of cases/controls/total N, matching factors, adjusting factors, and measures of effect and confidence intervals. Table 1 contains characteristics of studies included in the meta-analysis. One investigator performed the data extraction and again verified the data to check for inconsistencies. The corresponding author was contacted in instances when more information was required from the original publication to calculate the appropriate measures of effect for the meta-analysis (Weisskopf, Morozova et al. 2009). We attempted to include other studies; however, contacting the corresponding author to obtain the relevant information required for the meta-analysis proved unsuccessful in these cases.

3.3.2 Statistical Analysis

The reviewed studies measured self-reported exposure to pesticides (and a panel assessment of industrial hygienists in one study) by duration, frequency, concentration, or class of pesticide, depending on the study. In the case-control studies, cases were matched to controls by age and sex. Results were reported as ORs with 95% confidence intervals (CIs) for the majority of studies. Two cohort studies and two case-control studies provided relative risks and one cohort study provided standardized mortality ratios (SMRs), although ORs were calculated by the statistical software for inclusion in the meta-analysis. Three studies presented measures of effect adjusted for confounders. The weighted average estimate of the effect of pesticide exposure on ALS across the included studies served as our summary effect estimate.

Heterogeneity of studies was assessed by calculating both Q and I^2 statistics. The Q statistic is a standardized measure yielding the weighted sum of squares although it does not provide any information regarding the degree of heterogeneity (Thompson and Sharp 1999). Heterogeneity was considered statistically significant in our meta-analysis by a Q statistic *p*-value of <0.1 (Higgins and Thompson 2002). The I^2 statistic is used to determine the extent of true variability. An I^2 statistic of 25, 50, or 75 indicates low, medium, or high heterogeneity, respectfully (Higgins and Thompson 2002). Where evidence was found for heterogeneity, a random effects model was employed to pool study specific estimates. A fixed effect model was carried out for any meta-analyses in which evidence was found against heterogeneity ($I^2 \leq 25\%$). Sex was evaluated separately as a potential source of heterogeneity between studies.

A funnel plot was visually assessed to evaluate potential publication bias among studies (Sterne and Egger 2001). The x-axis contains the log of the ORs while the y-axis contains the standard error (SE) of the log of ORs. The presence of publication bias is determined by an asymmetrical

plot. Comprehensive Meta-Analysis software was used to conduct all analyses (Borenstein 2005).

3.4 RESULTS

3.4.1 Description of Studies

The meta-analysis included two prospective cohort studies and nine retrospective case-control studies. The studies varied by geographic location, exposure characterization, and measure of effect. Depending upon the study, ALS was diagnosed according to El Escorial Criteria, standard diagnostic criteria as described in detail, or identified by death certificates (ICD-9 code 335.2 or ICD-10 code G12.2). One study did not specify ALS diagnostic criteria (Savettieri, Salemi et al. 1991). Studies using standard diagnostic criteria had been conducted prior to the publication of El Escorial criteria in 1994. MND was diagnosed according to standard diagnostic criteria by two studies and by the presence of pure motor symptoms, a progressive course, and no signs of polyneuro-pathia by one study (Granieri, Carreras et al. 1988; Gunnarsson, Bodin et al. 1992; Chancellor, Slattery et al. 1993).

The random effects OR summary estimates found evidence for the association of exposure to pesticides and risk of ALS among all cases (OR=1.85, 95% CI: 1.27-2.71). Evidence was also found through a fixed effect model for exposure to pesticides and ALS among male cases (OR=1.88, 95% CI: 1.36-2.61) compared to controls (Figure 3). Conversely, no relationship was found between female cases exposed to pesticides and risk of ALS (OR=1.31, 95% CI: 0.69-

2.47) compared to controls, in a fixed effect model (Figure 4). The study specific ORs were considered heterogeneous at $p<0.10$.

A total of 1,115,901 participants (2,432 cases and 1,113,472 controls) were included in the analysis of the 11 studies. The sample sizes ranged from 123 to 952,728 participants among the studies. Controls consisted of individuals from the population (Gunnarsson, Bodin et al. 1992; Govoni, Granieri et al. 2005; Bonvicini, Marcello et al. 2010), community (Chancellor, Slattery et al. 1993; McGuire, Longstreth et al. 1997), hospital (Granieri, Carreras et al. 1988), or acquaintances (Deapen and Henderson 1986; Savettieri, Salemi et al. 1991). Morahan et al.'s study involved a combination of community, spouse, acquaintance, and relative controls (Morahan and Pamphlett 2006). Death certificates were consulted for verification of ALS mortality in the cohort studies (Burns, Beard et al. 2001; Weisskopf, Morozova et al. 2009). Age and sex-matched controls were used by most studies. Results were adjusted for by age, education, and year among other potential confounders, in three studies (McGuire, Longstreth et al. 1997; Burns, Beard et al. 2001; Bonvicini, Marcello et al. 2010).

3.4.2 Pesticide Exposure and ALS

Exposure history was obtained through self-report by the majority of the studies; although two studies consulted death certificates while another involved a panel assessment. Only one study obtained information related to exposure to a specific chemical or pesticide name; it investigated occupational exposure to the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) (Burns, Beard et al. 2001). Results were reported as ORs with 95% CIs for most of the studies or were converted into ORs as previously mentioned. ORs for pesticide exposure among ALS cases and controls are demonstrated by the Forest plots in Figure 2.

The result of the Q-test for all cases (Q=24.04, df=8, p=0.003) was heterogeneous indicating the effect size varied among studies and a random effects model was appropriate. The Q-statistic was not heterogeneous for men (Q=2.86, df=5, p=0.721) or women (Q=0.67, df=2, p=0.716) demonstrating that the studies share a common effect size and a fixed effect model should be used. The I^2 statistics for the main analyses were 66.72 for the overall model (men and women), 0.00 for men, and 0.00 for women indicating high and no heterogeneity, respectively.

3.4.3 Publication Bias

No evidence of publication bias was suggested by the funnel plots for any of the analyses as the studies were all symmetrical around the mean (data not shown).

3.5 DISCUSSION

The significant relationship of exposure to pesticides and risk of ALS as observed in our meta-analysis is an important finding. Overall, evidence was found for the association of exposure to pesticides and ALS among all cases and male cases compared to controls.

Studies conducted through May, 2011 were included in this quantitative meta-analysis investigating the association between pesticide exposure and risk of ALS. Exposure to class of pesticide (i.e. herbicide, fungicide, insecticide) was examined by only two of the studies and only one of them to a specific chemical, and was unable to be assessed by the meta-analysis due to the small number of studies. Therefore a gap identified in this field of research is better quantification of the precise type of pesticide, the active ingredient, and the introduction of

feasible monitoring of blood or urine analysis for better dose estimation. In addition, some studies provided duration of pesticide exposure; however, there were not enough studies with similar exposures to be combined in a stratified meta-analysis.

Heterogeneity across studies as demonstrated by the Q statistic ($p<0.1$) is likely due to differences between studies such as low power, methodology of pesticide exposure, study design or study population. The Q-statistic is not a reliable estimate of heterogeneity when a small number of studies are included in the meta-analysis. It is possible that with the addition of more (and larger) studies a stronger association may be detected. As expected, medium to high heterogeneity was indicated for the overall analysis of men and women by an I^2 statistic of 66.72. The analyses of men and women separately produced an I^2 of 0.00 indicating no heterogeneity between studies. One explanation for this may be the weighting of studies as reflected by the inverse of the study's variance. In a fixed effect model, the size of a study factors into its weight. The separate analyses for men and women also resulted in a T^2 (the between-studies variance) of 0.00; therefore, requiring the use of a fixed-effect analysis.

A fixed effect model was used in the absence of heterogeneity. When necessary, a random effects model was used to account for any heterogeneity between studies. We failed to find an association between pesticide exposure and risk of ALS in female cases compared to controls. This may be due to the small number of studies (n=3) and a small number of women in our analysis; therefore, resulting in a lack of power to detect an association. This may indicate that men are more likely to be occupational exposed to pesticides and for longer periods of time than women.

3.5.1 Comparison with previous research

No meta-analyses of pesticide exposure and ALS have been conducted to date. Reviews and epidemiological studies investigating the relationship between pesticide exposure and ALS have produced conflicting results. Some authors have reported an association (McGuire, Longstreth et al. 1997; Govoni, Granieri et al. 2005; Morahan and Pamphlett 2006; Bonvicini, Marcello et al. 2010), others have found non-significant increases (Deapen and Henderson 1986; Savettieri, Salemi et al. 1991), and still others have failed to replicate findings of the association of pesticide exposure and ALS (Granieri, Carreras et al. 1988; Chancellor, Slattery et al. 1993). Presently, only a small number of epidemiological studies have been carried out to investigate the relationship between exposure to pesticides and ALS development. The study designs have varied and have included case-series, case-control studies, and only a few prospective cohort studies. Controls selected for case-control studies have not always been population-based, which limits the representativeness of the results.

In addition, epidemiological studies conducted thus far have failed to report specific pesticide types and no epidemiological studies have attempted to obtain adequate exposure assessments through the use of blood samples or biomarkers, such as blood cholinesterase (ChE activity) and urinary metabolites. It may be possible, however, to draw a correlation between results of farming or toxicology studies measuring pesticide concentrations and those of epidemiological studies. For example, an exposure study carried out to evaluate exposure to glyphosate, a common herbicide used in farming, among farm families in South Carolina and Minnesota found an average urine concentration among farmers on application day of 3.2 ppb (parts-per-billion) (McKinney 2007). Following pesticide application, the concentration decreased. This is considerably lower than the lowest no-effect level as determined by the EPA (175 ppm)

(McKinney 2007). This study (as well as other exposure studies) provides valuable information regarding the level of pesticides to which farmers are potentially exposed.

Exposure has primarily been obtained through self-report; however, McGuire et al.'s study also incorporated a panel assessment to serve as a comparison. This was a useful implementation as it identified differences between exposure levels for both cases and controls. The type and magnitude of pesticide exposure is not usually obtained or reported. This is likely because self-report is not always an accurate measure of exposure; although years worked, number of hours exposed, and pesticides exposed to would not be difficult to additionally ask of participants. Therefore, the gold standard for future epidemiological studies investigating the association of pesticide exposure and risk of disease would be to obtain a thorough exposure assessment from multiple sources.

3.5.2 Strengths and Limitations

Our overall analysis as well as our analysis of men was fortunate to have a large sample size which allowed for sufficient power to detect an effect of exposure to pesticides and risk of ALS. Two studies examined pesticide exposure by class, duration, or intensity; however, a meta-analysis of these subgroups was not possible due to the small number of studies available (McGuire, Longstreth et al. 1997; Morahan and Pamphlett 2006). Excluding gardening in our analysis helped to eliminate the potential confounding effect of hobby-related exposures. Most studies used age- and sex-matched controls to alleviate potential confounding effects.

A limitation of our study is the possibility of publication bias from the literature search limits, from accessing only one database, and from the inclusion of only studies in the English language. However, the funnel plots were symmetric and publication bias does not appear to

have significantly affected the positive association found between pesticide exposure and ALS among all ALS participants (men and women) and men.

We must also take into account the limitations of the primary study designs included in the meta-analysis. In general, those who participate in research studies may be different than those who do not participate. A number of biases may be present within the case-control and prospective cohort study designs such as bias involved with self-reported exposure which may overestimate risk estimates. This is particularly important in retrospective studies as exposure assessment is conducted in an indirect manner. In addition, recall bias may play a role in that cases may more accurately remember exposures or information as compared to healthy controls.

The potential relationship between pesticide exposure and ALS has been difficult to establish as most studies have failed to obtain details regarding pesticide class (insecticide, herbicide, fungicide, etc.), chemical name, or duration of exposure. In our analysis, the majority of studies reported exposure to agricultural occupational chemicals but did not specify the chemicals or jobs involved. Categorizing subjects by level or duration of exposure (i.e. low, high, long-term, etc.) is helpful, although a meaningful conclusion cannot be made if the number of subjects in each group is too low as is the case among women with occupational exposure to pesticides. Grouping all pesticide classes together may dilute the effect of one class and result in a lack of an association. At any rate, more information should be provided regarding exposure categories reported by studies. The chemical composition of pesticides may not be known but commonly used brand names or uses of specific pesticides could be provided in the questionnaire or interview to better identify occupational and environmental exposures. In addition, questionnaires can discriminate by class of pesticide although this would be problematic for

some agricultural workers who may be exposed to multiple classes, at different times of the year, through different routes of exposure, and for different durations.

Misclassification is also a concern when occupational groups such as farming, combine various job titles regardless of exposure. Furthermore, the group "farmers" includes a number of different types of farmers such as soybean, livestock, corn, etc. Awareness of job exposures is necessary before grouping into occupational categories. Job exposure matrices (JEM) are also very valuable in identifying and quantifying occupational exposures and should be incorporated in future studies. Multi-site studies or collaborations between different states, countries, or universities would be an excellent way to improve sample size and power. These implications serve as only a starting point from which to expand future research. Studies included in our meta-analysis provided these details in some, but not all instances.

3.6 CONCLUSIONS

After examining all related articles through May, 2011, the meta-analysis found a significant relationship between exposure to pesticides and risk of ALS among all cases (men and women) and men compared to controls. Future research should focus on more accurate exposure measurement, the use of Job Exposure Matrices (JEM), and the inclusion of biological specimens. In addition, protective equipment should be worn by workers and during household use of pesticides to help circumvent any potential exposures and to prevent "take-home" exposures to others.

In conclusion, more research must be conducted to determine whether an association truly does exist between suspected pesticide exposure and risk of ALS. ALS is a debilitating and devastating disease, and one which certainly is deserving of additional research.

3.7 TABLES AND FIGURES

Table 3-1. Characteristics of Studies Included in the Meta-Analysis

Author, location of study	Year	Study design	Source of controls	ALS/MND diagnostic criteria	Pesticide exposure source	No. of cases/ controls (Total N)	Matching factors	Adjusting factors
Bonvicini, Italy (Bonvicini, Marcello et al. 2010)	2010	Case-control	Population controls (directory of residents)	ALS (El Escorial criteria)	Occupational pesticide exposure *>= 6 months*	41, 82 (N=123)	Age, sex	Education
Burns, U.S. (Burns, Beard et al. 2001)	2001	Cohort study	Deaths identified from cohort of Dow Chemical Company workers	ALS (ICD-8 348.0)	Occupational herbicide exposure to (2,4-dichlorophenoxyacetic acid) for varying durations of time (1.3, 1.8, or 12.5 years)	1517, 40,600 (N= 42,117)	Sex	Age, year
Chancellor, Scotland (Chancellor, Slattery et al. 1993)	1993	Case-control	Community controls	MND standard diagnostic criteria (SALS: multiple spinal level upper and lower motor neuron signs)	Occupational pesticide exposure *>=12 months*	103, 103 (N=206)	Age, sex	None reported
Deapen, U.S. (Deapen and Henderson 1986)	1986	Case-control	Acquaintance controls	ALS (patient registries from ALS Society)	Occupational pesticide exposure: *Long-term exposure*	518, 518 (N=1036)	Age, sex	None reported
Govoni, Italy (Govoni, Granieri et al. 2005)	2005	Case-control	Population controls	ALS (El Escorial criteria)	Farming occupation with exposure to agricultural chemicals: *N/A*	91, 159,949 (N= 160,040)	Not specified	None reported
Granieri, Italy (Granieri, Carreras et al. 1988)	1988	Case-control	Hospital controls	MND (based upon clinical findings of PMA, PBP and ALS)	Agricultural and forestry occupations; Agricultural chemical substances: *continuous occupational exposure*	70, 216 (N=286)	Age, sex, same period of hospital admission as cases (± 40 days), residency	None reported

Table 3-1 continued.

Author, location of study	Year	Study design	Source of controls	ALS/MND diagnostic criteria	Pesticide exposure source	No. of cases/ controls (Total N)	Matching factors	Adjusting factors
Gunnarsson, Sweden (Gunnarsson, Bodin et al. 1992)	1992	Case-control	Population controls	MND (pure motor symptoms, progressive course, no signs of polyneuro-pathia) ALS (LMN symptoms in at least 2 regions and 2 UMN symptoms within 3 years after onset)*	Male occupational exposure to pesticides and insecticides: *N/A*	92, 372 (N=464)	Age	None reported
McGuire, U.S. (McGuire, Longstreth et al. 1997)	1997	Case-control	Community control	ALS [Progressive MND affecting both UMN and LMN (ALS)*, and progressive muscular atrophy and progressive bulbar palsy (variants of ALS)]	*Ever* exposure to agricultural (agr.) chemical exposure; *Low/high to no* agr. chemical; *Ever* exposure to pesticides; *Low/high to no* pesticide exposure; *Ever* pesticide exposure; *Ever* exposure to other pesticides; *Ever* exposure to insecticides; *Low/high* to *no* insecticide exposure; Exposure to agr. chemicals *<3 years and >3 years*; Agr. exposure due to accident/spill (excess exposure by self-report);	174, 348 (N=522)	Age, sex	Age, education

* LMN=lower motor neurons, UMN=upper motor neurons

Table 3-1 continued.

Author, location of study	Year	Study design	Source of controls	ALS/ MND diagnostic criteria	Pesticide exposure source	No. of cases/ controls (Total N)	Matching factors	Adjusting factors
Morahan, Australia (Morahan and Pamphlett 2006)	2006	Case-control	Community, spouse, acquaintance and relative controls	ALS (probable or definite modified El Escorial criteria)	Herbicide/pesticide exposure *ever, occasional, and regular*; Farming herbicide/pesticide exposure *ever, occasional, and regular*; Exposure to industrial herbicide/pesticide *ever, occasional, and high dose;*	179, 179 (N=358)	Age, sex, ethnicity	None reported
Savettieri, Italy (Savettieri, Salemi et al. 1991)	1991	Case-control	Acquaintance controls	ALS (Did not report diagnostic criteria)	Agricultural chemicals: *Continual*	46, 92 (N=138)	Age, sex, place of residence (urban/rural), SES	None reported
Weisskopf, U.S. (Weisskopf, Morozova et al. 2009)	2009	Cohort	Deaths identified from CPS-II cohort of ACS	ALS (ICD-9 335.2, or ICD-10 G12.2 revision)	Pesticides/Herbicides: *Duration not reported*	1,115 951,613 (N= 952,728)	Not specified	None reported

1

Table 3-2. Results for Meta-Analyses of Studies, Overall and by Sex

		Effect size and 95% confidence interval			Heterogeneity		
Model	**Population**	**Number of Studies**	**Odds Ratio**	**95% CI**	**P-value**	**Q-value**	I^2
Random	All	9	1.85	1.27-2.71	0.003	24.04	66.72
Fixed	Men	6	1.88	1.36-2.61	0.721	2.86	0
Fixed	Women	3	1.31	0.69-2.47	0.716	0.67	0

Abbreviations: CI, confidence interval.

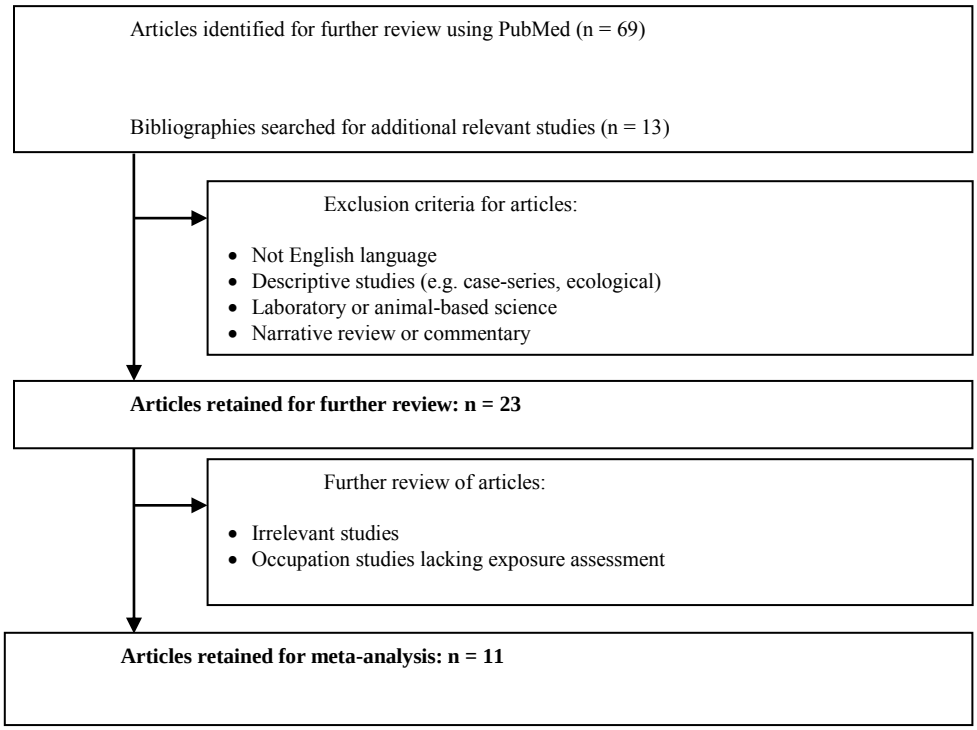

Figure 3-1. QUORUM Summary Diagram of Studies Included in Meta-Analysis

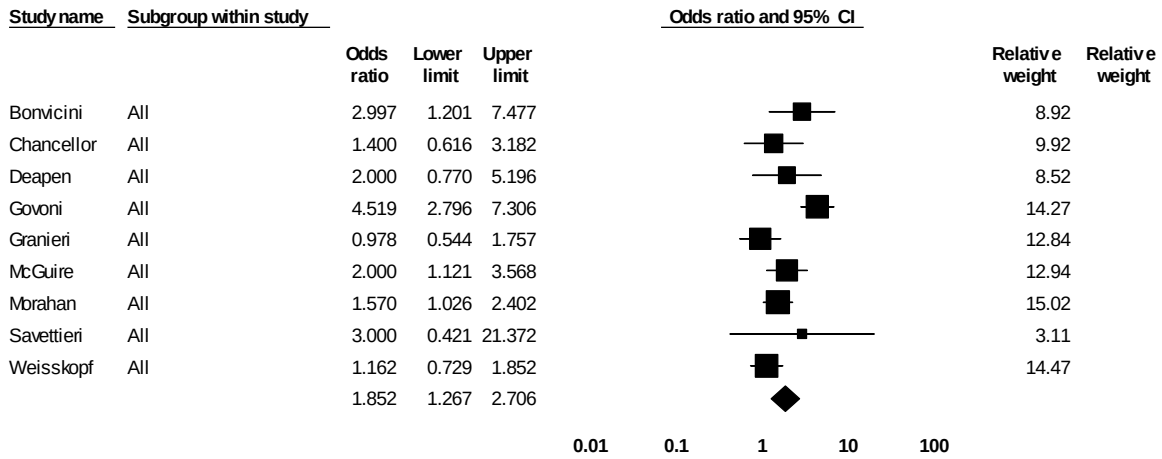

Figure 3-2. Random effects model of pooled odds ratios (ORs) with corresponding 95% confidence intervals (CIs) for pesticide exposure and ALS among all (men and women)

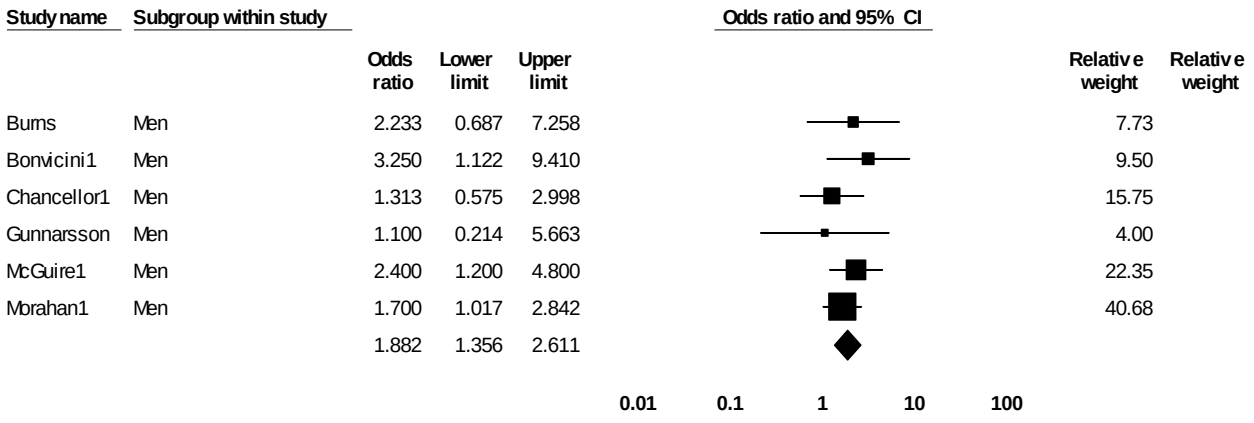

Figure 3-3. Fixed effect model of pooled odds ratios (ORs) with corresponding 95% confidence intervals (CIs) for pesticide exposure and ALS among men

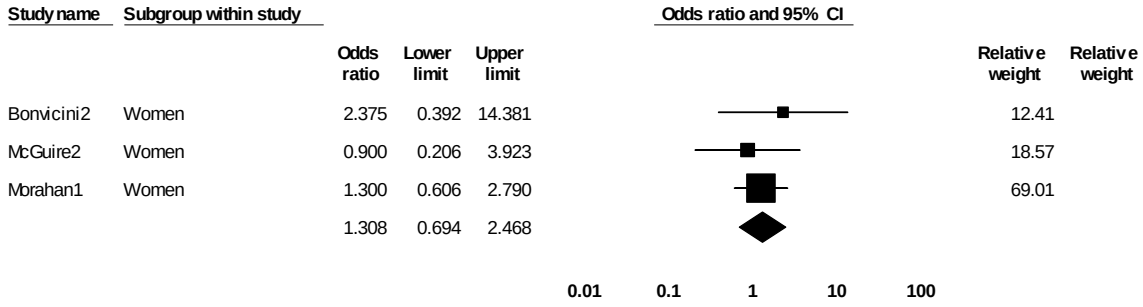

Figure 3-4. Fixed effect model of pooled odds ratios (ORs) with corresponding 95% confidence intervals (CIs) for pesticide exposure and ALS among women

4.0 THE ASSOCIATION OF PERSONAL FACTORS AND ENVIRONMENTAL AND OCCUPATIONAL EXPOSURES AND THE RISK OF AMYOTROPHIC LATERAL SCLEROSIS

Angela M. Malek, Ph.D.[1]

Aaron Barchowsky, Ph.D. [2]

Robert Bowser, Ph.D. [3]

Terry Heiman-Patterson, M.D. [4]

David Lacomis, M.D. [5]

Sandeep Rana, M.D. [6]

Ada Youk, Ph.D. [7]

Evelyn O. Talbott, Dr.P.H, M.P.H. [1]

[1] Department of Epidemiology, University of Pittsburgh,
Graduate School of Public Health, Pittsburgh, PA 15261

[2] Department of Environmental and Occupational Health, University of Pittsburgh,
Graduate School of Public Health, Pittsburgh, PA 15261

[3] Department of Pathology, University of Pittsburgh,
College of Medicine, Pittsburgh, PA 15261

[4] Department of Neurology, Drexel University, School of Medicine,

School of Medicine, Philadelphia, PA 19107

[5] Department of Neurology, University of Pittsburgh,
School of Medicine, Pittsburgh, PA 15261

[6] Department of Neurology, Temple University School of Medicine,
Philadelphia, PA 19122

[7] Department of Biostatistics, University of Pittsburgh,
Graduate School of Public Health, Pittsburgh, PA 15261

Manuscript in preparation

4.1 ABSTRACT

Background: Environmental and occupational exposures are implicated as risk factors for Amyotrophic lateral sclerosis (ALS) although no causal relationships have been proven.

Methods: Our goal was to examine the association of personal factors and environmental and occupational exposures and the risk of ALS. A case-control study of 66 ALS cases and 66 age, sex, and race-matched controls was conducted in Western Pennsylvania (Pittsburgh region) and the greater Philadelphia area over a 24-month period. A detailed questionnaire consisting of lifetime occupation, vocation and avocation exposures, as well as personal lifestyle factors was administered by personal interview. Descriptive analyses were conducted and odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using conditional logistic regression.

Results: After controlling for smoking and education, occupational exposure to metals was associated with increased risk of ALS (OR=3.65, 95% CI: 1.15, 11.60, p=0.03). Occupational exposure to pesticides was also related to increased ALS risk after controlling for smoking and education (OR=6.50, 95% CI: 1.78, 23.77, p=0.005). Risk of ALS did not differ from those without exposure to organic solvents, aromatic solvents, or electrical or electronic equipment or machinery.

Conclusion: The public health significance of this study is that occupations involving metal and pesticide exposure may be at greater risk of ALS. Future research should involve more accurate exposure assessment and occupational history.

Key words: amyotrophic lateral sclerosis, occupational exposures, metals, pesticides, epidemiology

4.2 INTRODUCTION

Amyotrophic lateral sclerosis (ALS) or Lou Gehrig's disease is the most common motor neuron disease (MND) in adults and is characterized by progressive degeneration of both the upper and lower motor neurons in the brain and spinal cord, ultimately resulting in death (Borasio and Miller 2001). The global incidence of ALS is approximately 1-3 cases per 100,000 annually, and the prevalence is about 6 cases per 100,000 (Mitchell and Borasio 2007; Alliance 2009; Migliore and Coppede 2009). ALS increases in incidence in those over fifty years of age until the age of 75 (Kamel, Umbach et al. 2005; Alliance 2009; Migliore and Coppede 2009). In general, trends over time indicate that mortality from ALS has been increasing in the U.S., Israel, Europe, and Japan (Kelly 2001). Men develop ALS at 1.5 times the rate of women although the inequality seems to balance out among the two sexes in older age (Kamel, Umbach et al. 2005). After onset of ALS has occurred, the median survival is 2-4 years (Borasio and Miller 2001). The only currently approved drug treatment for ALS (Rilutek) extends life by about 3 months. No other treatment exists to treat or prevent this devastating disease.

Male sex and age are the only known risk factors for ALS; however, an environmental etiology for ALS has been supported by epidemiologic studies and is suggested by the gender discrepancy (Nelson 1995; Strong and Rosenfeld 2003). Many occupational and personal risk factors have also been investigated over the years, some of which have produced conflicting results. Overall, there have been more than 30 peer-reviewed case-control epidemiological studies and at least 7 prospective epidemiological studies of ALS. Since the 1970's, the majority of studies have found an association between exposure to metals such as lead, mercury or others, and risk of ALS; 17 studies found a relationship (Campbell, Williams et al. 1970; Felmus, Patten et al. 1976; Rosati, Pinna et al. 1977; Schwarz 1977; Conradi, Ronnevi et al. 1978; Roelofs-

Iverson, Mulder et al. 1984; Gresham, Molgaard et al. 1986; Provinciali and Giovagnoli 1990; Armon, Kurland et al. 1991; Gunnarsson, Bodin et al. 1992; Chancellor, Slattery et al. 1993; Strickland, Smith et al. 1996; Vinceti, Guidetti et al. 1996; McGuire, Longstreth et al. 1997; Longnecker, Kamel et al. 2000; Kamel, Umbach et al. 2002; Armon 2004) while others did not (Gresham, Molgaard et al. 1986; McGuire, Longstreth et al. 1997; Vinceti, Guidetti et al. 1997; Gait, Maginnis et al. 2003). The relationship between exposure to pesticides, agricultural occupations, and living in a rural area, and ALS has been inconsistent as only about half of the epidemiologic studies have found an association (Giagheddu, Puggioni et al. 1983; Granieri, Carreras et al. 1988; Gunnarsson, Lindberg et al. 1991; McGuire, Longstreth et al. 1997; Qureshi, Hayden et al. 2006). Several studies have not found an association of pesticide exposure, agricultural occupations, or living in a rural area and ALS (Deapen and Henderson 1986; Armon, Kurland et al. 1991; Savettieri, Salemi et al. 1991; Gunnarsson, Lygner et al. 1996). The association of occupational electromagnetic field exposure and ALS has also been investigated, with 9 of 10 published reports indicating a positive relationship (Li and Sung 2003).

This important research aims to further elucidate the epidemiological evidence for ALS etiology by investigating the relationship of environmental and occupational exposures in two Pennsylvania locations (W. Pa and greater Philadelphia area). W. Pa is known for its long history of steel making. W. Pa is also home to several National Priorities List (NPL) sites, landfills, chemical plants, coalmines, coal-fired power plants, with Pittsburgh being the largest coke producer. However, we also included ALS patients from the Philadelphia area to create a more regional study population and facilitate rate of patient enrollment. A number of industrial sites, Superfund sites, oil refineries, metals fabricators, chemical plants, and power generating stations are located in this region.

4.3 METHODS

4.3.1 Study Population

The "Risk Factors for ALS" study was approved by the University of Pittsburgh Institutional Review Board, Allegheny General Hospital Institutional Review Board, and Drexel University College of Medicine Institutional Review Board. Written informed consent was obtained from all participants. The specific aim was to evaluate the association of personal risk factors (physical activity, caffeine intake, alcohol, family history of neurological disease, etc.) and environmental and occupational exposures (metals, pesticides, solvents, electrical exposure, relevant jobs, etc.), and the risk of ALS.

Sporadic ALS patients were recruited between December 2008 and July 2010, from three neurology clinics with ALS centers; two in Pittsburgh, PA and one in Philadelphia, PA. ALS was diagnosed by board certified neurologists according to the World Federation of Neurology El Escorial criteria and included possible, probable, and definite ALS cases (Brooks 1994). Neurologists used data from the neurologic exam, clinical history, electrophysiological tests, laboratory studies, and imaging studies as available to diagnose patients. Cases and controls were required to live in one of the following study counties in Western PA: Allegheny; Armstrong; Beaver; Butler; Washington; or Westmoreland, or in the greater Philadelphia area: New Castle County, Delaware (DE); Union or Somerset County, New Jersey (NJ); Montgomery, Philadelphia, or Sussex County, PA for at least one year prior to the study. These counties were chosen for our study population because they are in close proximity to the larger metropolitan areas of Pittsburgh and Philadelphia, both of which have ALS Centers that provide medical treatment and therapy for patients as well as conduct research. Patients often travel from the

surrounding counties to receive medical care from University of Pittsburgh Medical Center, Allegheny General Hospital, Drexel University College of Medicine, as well as the other large medical centers in the area. This is especially true for disorders such as ALS, where a specialist may be required.

Cases and controls were excluded from study participation for any of the following conditions: first, second, or third degree blood relative in whom ALS had previously been diagnosed, history of travel to Guam or other islands of the Mariana group, history of poliomyelitis/post-polio syndrome, Parkinson's disease, parkinsonism, Alzheimer's disease, or dementia. As our study investigated risk factors associated with sporadic ALS (SALS) alone, cases or controls with a family history of ALS were excluded. Cases or controls who had traveled extensively to the western Pacific Islands of Guam, West Papua, the Kii Peninsula of Japan, or the other Mariana group islands were also excluded to control for the possibility of the high incident variant of ALS. The neurological conditions listed above were exclusion criteria as they are related to ALS. Cases and controls were also required to speak English.

The two ALS centers in Pittsburgh saw an average of 75 new patients (total) per year while the ALS center in Philadelphia saw about 45 new patients per year. All sites combined saw a total of n=150 for the study period. Philadelphia joined the study later, and as a result, did not have the opportunity to contact and enroll all of their potentially eligible patients. Letters were sent to ALS patients by their neurologist to inform them of the study and ask them to return a card to indicate their interest in participating. In addition, patients were informed in person about the study at ALS clinics by their neurologist. Two research coordinators were present in the waiting room to conduct the interview after interested patients were screened and consented. Approximately 30% of ALS patients were ineligible to participate because they had familial

ALS or did not live in one of the designated study counties. A total of 106 patients were contacted about the study of which 78 participated, with a response rate of 73.6%. Only 66 of the 78 cases who completed a questionnaire were included in the study due to a lack of matched controls (57 from W. Pa and 9 from Philadelphia). The majority of patients were enrolled in the first year of the study. Eligible patients who did not participate were not interested, changed their mind, died prior to being informed of the study or having the chance to participate, or were lost to follow-up.

Eligible controls were required to be free of ALS. All controls were matched to cases by 1:1 matching on age of case's first ALS symptoms ($\pm$ 5 years), sex, race, and region (W. Pa or the greater Philadelphia area) and included both hospital and population controls. Hospital controls were recruited from neurology office waiting rooms in Pittsburgh between 2009 and 2011, over an 18-month period. The same inclusion and exclusion criteria for cases applied to controls. Potential matched-population controls were identified through an online consumer marketing database, www.InfoUSA.com, for the greater Philadelphia area (n=2,000) (Infogroup 2011). A random sample of 10 potential controls was selected for each case from InfoUSA lists (n=133 or n=134) using a random number generator (Urbaniak 1997-2008). Therefore, ten matched potential controls were contacted by mail for each case to inquire about their interest in participating in the study. A subsequent mailing took place if a response was not received. Upon lack of a response following the second mailing, a new control was randomly selected from the original sample in the same manner excluding any previously contacted controls. If a mailing was returned due to an incorrect address, a new control was selected and contacted as previously outlined. The response rate for eligible controls was 70.3% for W. Pa and 4.7% for the greater Philadelphia area mailing.

4.3.2 Exposure Assessment

A modified version of the ALS Consortium of Epidemiologic Studies (ACES) ALS Risk Factor Questionnaire (ALSRFQ) was administered by personal interview (ACES 2005). This detailed questionnaire consisted of questions on lifetime occupation, residential history, vocation and avocation exposures, pesticide use, as well as personal lifestyle factors. The occupational history included all jobs held since the age of 19 for at least two years. Most recent occupation was examined prior to date of first symptoms for cases and the corresponding age for controls, as controls were matched to cases by case's age at first symptoms. Participants provided a history of their job title, industry, description of the job, and the calendar years worked at the job. The 1980 U.S. Census industrial and occupational classification coding system was consulted to code the occupations of participants according to six occupational categories which included: 1) managerial and professional specialty; 2) technical, sales, and administrative support; 3) service; 4) precision production, craft, and repair; 5) operators, fabricators, and laborers; and 6) farming, forestry, and fishing (Census 1982). A seventh category was added for full-time homemakers.

Also obtained was self-reported occupational exposure for 21 agents grouped under the 7 main agent categories of: metals (lead and mercury); pesticides (insecticides, herbicides, fungicides and fumigants); organic/chlorinated solvents (paint strippers, adhesives, degreasers and other cleaning agents, dry cleaning agents, and dyes or printing inks); aromatic solvents, petroleum, and rubber (solvents such as toluene and xylene, mineral spirits or white spirits, varnishes, oil-based paint, paint thinners, cutting, cooling, and lubrication oils); diesel and gasoline fuel; antifreeze and coolants; and electrical and electronic equipment and machinery including electromagnetic fields such as power lines or transformer stations. Occupational exposures included any agent(s) participants had been exposed to at least 10 or more times while

working on any job (since the age of 19). The calendar year first exposed, total number of years exposed, and the number of days per year exposed was obtained for each agent. For analysis purposes a new variable, lifetime days of exposure, was calculated for each agent by multiplying the number of years exposed by the number of days (per year) exposed. Lifetime days of exposure was then categorized as ≥ 2,000 days, 400-1,999 days, or 1-399 days according to categorization previously published by an occupational study of ALS (Fang, Quinlan et al. 2009).

4.3.3 Statistical Analysis

Descriptive analyses were conducted using the chi-square test and paired t-test. The Wilcoxon signed rank test was used for non-normally distributed data. The risk of ALS was evaluated by odds ratios (ORs) and corresponding 95% confidence intervals (CIs) derived from conditional logistic regression models. Ever smoking was characterized as smoking 100 or more cigarettes in a lifetime. Pack years was calculated by the number of packs (or proportion of a pack) of cigarettes smoked per day multiplied by the number of years smoked. In instances where someone worked in more than one occupation they were counted separately in each industry and occupation category. Analyses were stratified by occupational exposures to account for the possibility of interactions.

Forward stepwise conditional logistic regression was conducted and included variables at entry that were significant at $p<0.05$ and removed those with $p<0.10$. Models adjusted for smoking (ever vs. never) and education (high school or less vs. more than high school) were fit. *P*-values of < 0.05 were considered statistically significant and two-tailed tests were used.

Statistical Package for the Social Sciences (SPSS) Statistics 18, version 18.0.0 was used to conduct all statistical analyses (IBM SPSS 2010).

4.4 RESULTS

4.4.1 Unpaired Sociodemographic Characteristics

Demographic characteristics of cases and controls are displayed in Table 1. The majority of cases and controls were male (68.2%), Caucasian or white race (98.5%), and from W. Pa (greater Pittsburgh region) (86.4%). The remaining cases and controls were from the greater Philadelphia area. Cases and controls were well-matched on age, race, sex and region. Age varied slightly among cases and controls for the following age groups: 45-54 years (19.7% to 25.8%), 64-74 years (27.3% to 22.3%), and 75 years and older (9.1% to 6.1%). In general, cases were somewhat older than controls.

High school, vocational or technical training, or less than a high school education was completed by nearly half of the cases (45.5%) and 40.8% of controls. Slightly fewer cases (54.6%) than controls (59.1%) had obtained further education following high school including attending some college up to a graduate degree. On average, cases were less educated than controls. The majority of cases and controls were married or cohabiting (81.8% and 75.8%, respectively).

4.4.2 Unpaired Personal and Environmental Risk Factors

Living on a farm or use of residential well water was obtained through participants' residential history. Nearly 80% of cases and 88% of controls had never lived on a farm, while three percent of controls and no cases lived on a farm for 1 to 3 years. More cases (6.1%) than controls (3%) had lived on a farm for 4 to 9 years. Living on a farm for 10 to 14 years (1.5%) as well as for 15 to 20 years (4.5%) was similar among cases and controls. Cases were the only group to have lived on a farm for more than 20 years (7.6%). This was the main difference found among cases and controls with who had lived on a farm. Residential well water was used by a larger proportion of cases and for longer periods of time compared to controls in all but one time period (unpaired p=0.03). Approximately half of all cases and 74.2% of controls had never used residential well water. More cases than controls used residential well water for 1 to 3 years and for 4 to 9 years. Residential well water use was similar among cases and controls for 10 to 14 years and for 15 to 20 years. Twice as many cases as controls used residential well water for 20 or more years (18.2% vs. 9.1%, respectively). Cases were more likely than controls to use residential well water for 1 to 3 years, 4 to 9 years, and more than 20 years. Residential well water use may be related to living on a farm as more cases lived on farms for two of these time periods.

Somewhat more cases (40.9%) than controls (36.4%) had never smoked cigarettes, while 59.1% of cases and 63.6% of controls had ever smoked, (defined as smoking at least 100 cigarettes). Among those who smoked, the majority (~60%) of cases and controls smoked for more than 20 years. More cases (12.8%) than controls (2.4%) smoked for 16 to 20 years. A similar proportion of cases and controls smoked for 1 to 5 years and 11 to 15 years. More controls than cases smoked for 6 to 10 years. More cases than controls smoked less than a pack

per day (51.3% and 47.6%, respectively). A similar number of cases (46.2%) and controls (47.6%) smoked 1 to 2 packs per day. Controls were more likely than cases to smoke more than 2 packs per day (4.8% vs. 2.6%). Overall, controls were more likely than cases to smoke; however, more cases smoked for longer periods of time.

A quarter of cases and 33.3% of controls did not drink alcohol prior to the reference date, defined as consuming at least one drink per month for 6 months or more. Of those who drank alcohol, cases were more likely to consume 1 to 19 drinks per month compared to controls (47.8% and 41.9%, respectively). However, more controls (58.1%) reported drinking 20 or more drinks per month compared to cases (34.4%). The frequency of drinking alcohol was categorized from once per month to 17 or more times per month. A small percentage of cases drank only once per month. More cases (30.6%) drank alcohol 2 to 4 times per month than did controls (22.7%). Controls were more likely to drink alcohol 5 to 8 times per month compared to cases (29.5% and 18.4%, respectively). The proportion drinking 9 to 16 times per month was similar for cases (16.3%) and controls (20.5%). Slightly more cases (28.6%) than controls (27.3%) drank alcohol 17 or more times per month. In general, more cases than controls drank alcohol. Although the number of times alcohol was drunk per month was similar for cases and controls, controls consumed more drinks per month ($\geq$ 20).

4.4.3 Demographic Characteristics and Personal Risk Factors

Demographic characteristics for paired data and conditional logistic regression models (ORs and 95% CIs) are summarized in Table 2. A similar number of cases and controls (n=42) completed further education following high school which included attending some college, obtaining an associate's degree, bachelor's degree, or a graduate degree.

Among those who smoked cigarettes, mean pack years was slightly higher for cases currently smoking (26.84 $\pm$ 23.04) compared to controls (24.82 $\pm$ 25.09) (p=0.80). Among former smokers, controls had a higher mean pack year (19.5 $\pm$ 6.36) than cases (16 $\pm$ 1.41) (p=0.70). Smoking $\geq$ 1 pack per day compared to smoking < 1 pack per day was comparable among pairs of cases and controls (OR=1.00, 95% CI: 0.32, 3.10). In general, cases smoked for a longer period of time than controls although both groups smoked a similar number of cigarettes per day. Alcohol consumption was also assessed and found no difference among case and control pairs drinking alcohol 17 or more times per month compared to those drinking less than 17 times per month (OR=0.67, 95% CI: 0.19, 2.36).

Residential well water use and living on a farm were also assessed. Use of residential well water for 15 years or longer compared to less than 15 years was not significantly different among cases and controls (OR=0.50, 95% CI: 0.05, 5.51). Living on a farm for 15 years or longer compared to living on a farm for less than 15 years was not significantly different in the conditional logistic regression. Overall, use of residential well water and years lived on a farm were similar for cases and controls.

4.4.4 Personal Risk Factors: Medical and Medication History

Personal risk factors such as medical history, trauma and electrical shock, physical activity, and military service, etc. are displayed in Table 3 by ORs and 95% CIs calculated by conditional logistic regression for paired cases and controls. No significant differences were found between cases and controls for any of the following conditions: hypothyroidism or Grave's disease (OR= 0.50, 95% CI: 0.09, 2.73), hyperthyroidism (OR=1.00, 95% CI: 0.06, 15.99), myocardial infarction or coronary thrombosis (OR=2.00, 95% CI: 0.50, 8.00), stroke or transient ischemic

attack (OR=0.02, 95% CI: 0.00, 1327.4), hypertension (OR=1.21, 95% CI: 0.60, 2.46), or hyperlipidemia (OR=1.73, 95% CI: 0.82, 3.63). No cases or controls reporting having been diagnosed with hyperparathyroidism. Tranquilizer and muscle relaxer use was similar among cases and controls (OR=0.71, 95% CI: 0.34, 1.48). Use of psychotherapeutic drugs was similar for cases and controls and saw no difference in the paired analysis. The proportion of cases and controls currently taking cholesterol-lowering medication (OR=2.00, 95% CI: 0.18, 22.06), who had received polio immunization (OR=1.60, 95% CI: 0.52, 4.89), or who had received spinal anesthesia at some point in their lives (OR=0.60, 95% CI: 0.22, 1.65) was also similar. In general, cases and controls were not statistically different with regards to medical history and medications taken.

4.4.5 Caffeinated Coffee and Diet Beverage Consumption

Cases drank caffeinated coffee for a longer period of time (years) than controls as found by the paired analysis (mean difference: 4.61 ± 13.68; p=0.045). No difference was found for diet soda or diet drink consumption among cases and controls in the paired analysis (p=0.18).

4.4.6 Trauma and Electrical Shock

No differences were found for cases and controls experiencing a head injury resulting in unconsciousness or requiring medical care (OR=0.87, 95% CI: 0.41, 1.82), or for experiencing a severe injury requiring medical attention (OR=0.63, 95% CI: 0.28, 1.38). Receiving severe electrical shocks affected an equal number of cases and controls (16.7%). Experiencing more than one severe electrical shock was not significantly different among matched pairs.

4.4.7 Physical Activity

A similar number of cases and controls participated in intercollegiate sports or were professional or semi-professional athletes or amateur competitive athletes (OR=1.11, 95% CI: 0.45, 2.73). Strenuous physical activity was defined as activity that makes a person breathe hard and was evaluated by mean number of hours per week exerted during the various age categories. No significant differences were found between cases and controls for any of the age categories of strenuous physical activity: 15 to 24 years (p=0.39); 25 to 34 years (p=0.94); 35 and 44 (p=0.31); and 45 years and older (p=0.54).

4.4.8 Military History

No significant differences were found for cases and controls who served in the military (OR=0.64, 95% CI: 0.25, 1.64). It is interesting to note that of those who served, almost all controls were male (94.4%) while only about three-fourths of cases were male (71.4%). A few cases (1.5%) and controls (6.1%) were involved in combat but there was no significant difference.

4.4.8.1 Family History of Neurological Disease

Table 4 presents biological family history of neurological disease for cases and controls. No differences were found for any type of family history of Parkinson's disease or Parkinsonism, Alzheimer's disease or dementia, thyroid disease, or any other diseases affecting the nervous system, among cases compared to controls. No siblings had been diagnosed with Parkinson's

disease, and most of the children were too young to develop Parkinson's disease, Parkinsonism, Alzheimer's disease, dementia, or thyroid disease.

4.4.8.2 Occupational Classification

Occupations of cases and controls were categorized according to the 1980 U.S. Census occupational coding (see Table 5). The 1980 coding was chosen due to the small number of cases and controls in each category. There were no significant differences for cases and controls among any of the occupational categories: managerial and professional specialty occupations (OR=1.40, 95% CI: 0.62, 3.15); technical, sales, and administrative support (OR=0.71, 95% CI: 0.34, 1.48); service occupations (OR=0.78, 95% CI: 0.29, 2.09); precision production, craft, and repair occupations (OR=0.88, 95% CI: 0.43, 1.79); or operators, fabricators, or laborers (OR=1.50, 95% CI: 0.42, 5.32). Only cases were employed in farming, forestry, or fishing (n=1) and as full-time homemakers (n=2).

4.4.8.3 Occupational Exposures

Table 6 presents self-reported occupational exposure characterized by agent of exposure for cases and controls by unpaired analysis, paired analysis, and conditional logistic regression. Those with occupational exposure to pesticides were found to have 3.17 times the risk of developing ALS compared to those without occupational exposure to pesticides (OR=3.17, 95% CI: 1.27, 7.93). Univariate paired analysis of pesticide exposure and risk of ALS was also significant (no. of pairs, 19 vs. 6) (p=0.02). Occupational exposure to electrical and electromagnetic equipment and machinery and electromagnetic fields was protective for cases

compared to controls in the paired analysis (no. of pairs, 11 vs. 27) (p=0.01) as well as in conditional logistic regression (OR=0.41, 95% CI: 0.20, 0.82). No significant differences between cases and controls were found for occupational exposure to metals (OR=0.89, 95% CI: 0.84, 4.24); organic and chlorinated solvents (OR=0.75, 95% CI: 0.38, 1.47); aromatic solvents, petroleum, and rubber (OR=1.13, 95% CI: 1.13, 95% CI: 0.57, 2.27); diesel or gasoline fuel (OR=0.77, 95% CI: 0.34, 1.75); or antifreeze and coolants (OR= 1.25, 95% CI: 0.49, 3.17). Overall, occupational exposure was associated with ALS in cases compared to controls. Occupational exposure to electrical and electromagnetic equipment and machinery and electromagnetic fields was found to be protective for ALS.

4.4.8.4 Occupational Exposures by Lifetime Days of Exposure

Self-reported occupational exposures by lifetime days of exposure (1 to 399 days, 400 to 1,999 days, and ≥ 2,000 days) characterized by agent of exposure were evaluated for cases and controls (see Table 7). Less than < 2,000 lifetime days of exposure to the various substances was compared to ≥ 2,000 lifetime days of exposure. No significant differences were found for lifetime days of exposure to any of the substances occupationally (metals; pesticides; organic or chlorinated solvents; aromatic solvents, petroleum and rubber; diesel and gasoline fuel; antifreeze and coolants; or electrical and electronic equipment and machinery or electromagnetic fields) among cases and controls. The majority of cases and controls with occupational exposure to diesel and gasoline; aromatic solvents, petroleum, and rubber; and electrical and electronic equipment or machinery reported ≥ 2,000 lifetime days of exposure. Organic and chlorinated solvents were also common occupational exposures, especially ≥ 2,000 lifetime days of exposure for both cases and controls, and 1-399 lifetime days of exposure for controls only. Cases

reported greater occupational lifetime days of exposure to metals and pesticides than controls in all but one category of lifetime days. Occupational exposure to antifreeze and coolants was somewhat similar among the various lifetime days of exposure categories and for both cases and controls.

4.4.8.5 Final Model

A final model using conditional forward stepwise logistic regression was conducted to evaluate the association of personal risk factors and occupational exposures and ALS. After controlling for smoking (ever vs. never) and education (less than or equal to high school vs. more than high school), cases were found to have significantly greater occupational exposure to metals (OR=2.71, 95% CI: 1.02, 7.22, p=0.047) and pesticides (OR=5.24, 95% CI: 1.65, 16.67, p=0.005) compared to controls. Occupational exposure to electrical or electronic equipment or machinery or electromagnetic fields was protective for cases (OR=0.28, 95% CI: 0.12, 0.66, p=0.004).

4.5 DISCUSSION

This study found an association of occupational exposure to metals and pesticides and higher risk of ALS after adjustment for smoking and education. Our findings confirm that of previous research suggesting a potential relationship between metal exposure and ALS (Felmus, Patten et al. 1976; Conradi, Ronnevi et al. 1978; Armon, Kurland et al. 1991; Chancellor, Slattery et al. 1993) and between pesticide exposure and ALS (McGuire, Longstreth et al. 1997; Kamel and

Hoppin 2004; Govoni, Granieri et al. 2005; Morahan and Pamphlett 2006; Bonvicini, Marcello et al. 2010). Our results suggest a possible link between occupations involving exposure to metals or pesticides and elevated risk of ALS.

The univariate analysis found a relationship between well water use and ALS in unpaired analyses, which would be interesting to explore further as many metals or exposures may be present in unregulated residential well water. The previously reported but inconsistent associations of electrical shocks, metal exposure, living near farms, smoking, physical activity, and military history, family history of neurological diseases and ALS were unable to be confirmed through our study.

As the etiology of ALS is currently unknown, previous findings associated with occupational and environmental exposures have been inconsistent. Possible reasons for this may include different classifications of occupations by U.S. Census coding or other methods, and varying definitions of exposures or occupations such as pesticides and farming. The majority of exposure assessment is obtained through self-report without verification or biological sampling. In addition, specific agents or chemicals are not always asked about by researchers and may not be known by participants.

Several limitations should be considered. Recall bias may be present with the use of a retrospective study design. Selection bias may also be a concern. A number of interested ALS patients completing the questionnaire were rejected because they did not meet the regional study criteria in W. Pa, and due to a lack of controls for the greater Philadelphia area. Our study was underpowered to assess any potential relationships in women or other racial/ethnic groups other than Caucasians or whites. Two different control populations were used, one of which was

outpatient-based. As a result of these limitations, the results are not generalizable to the general population.

The matched case-control design was an important asset to help account for the possibility of confounding among factors matched upon. Enrolling ALS patients from the two ALS centers in Pittsburgh captured the large majority of ALS patients in the area. Inclusion of the Philadelphia ALS center facilitated patient enrollment and permitted a more regional sample distribution throughout Pennsylvania. Furthermore, the addition of another study site helped to improve the overall sample size. Our sample of cases and controls was well-matched and participants were evenly distributed across the various age groups.

4.6 CONCLUSIONS

The relationship of occupational exposure to metals and pesticides and increased risk of ALS suggests that certain occupations may be associated with ALS. Future research should involve larger sample sizes, population-based controls, biomarkers, and more detailed occupational data. The use of job exposure matrices would provide more accurate exposure assessments thus improving the quality of available data.

4.7 TABLES

Table 4-1. Demographic characteristics of ALS cases and controls.

Table 4-1. Demographic characteristics of ALS cases and controls.

Demographic Characteristic	Cases (*n* = 66) No. (%)	Controls (*n* = 66) No. (%)	X^2	*p*
Sex				
Male	45 (68.2%)	45 (68.2)	N/A	N/A
Female	21 (31.2%)	21 (31.2%)		
Age (years)				
Mean age [b]	57.12 ± 13.23	56.38 ± 13.49	1.25	0.07**
18-44	12 (18.2%)	13 (19.7%)		0.87
45-54	13 (19.7%)	17 (25.8%)		
55-64	17 (25.8%)	17 (25.8%)		
65-74	18 (27.3%)	15 (22.3%)		
≥ 75	6 (9.1%)	4 (6.1%)		
Race				
Caucasian or White	65 (98.5%)	65 (98.5%)	N/A	N/A
African-American or Black	1 (1.5%)	1 (1.5%)		
Region				
Western Pennsylvania	57 (86.4%)	57 (86.4%)	N/A	N/A
Greater Philadelphia area	9 (13.6%)	9 (13.6%)		
Education				
Grade school (1-8 years)	4 (6.1%)	2 (3.0%)	3.81	0.70
High school (9-12 years)	20 (30.3%)	22 (33.3%)		
Vocational/technical training	6 (9.1%)	3 (4.5%)		
Some college	17 (25.8%)	17 (25.7%)		
Associate's degree	2 (3.0%)	6 (9.1%)		
College degree	11 (16.7%)	10 (15.2%)		
Graduate degree	6 (9.1%)	6 (9.1%)		
Marital status				
Single and Never Married	6 (9.1%)	6 (9.1%)	1.15	0.56
Married or Cohabiting	54 (81.8%)	50 (75.8%)		
Divorced or Widowed	6 (9.1%)	10 (15.2%)		
Smoking status				
Never	27 (40.9%)	24 (36.4%)	0.29	0.59
Ever (≥ 100 cigarettes)	39 (59.1%)	42 (63.6%)		
Amount				
< 1 pack per day	20 (51.3%)	20 (47.6%)	0.33	0.85
1-2 packs per day	18 (46.2%)	20 (47.6%)		
> 2 packs per day	1 (2.6%)	2 (4.8%)		
No. of years smoked				
1-5 years	7 (17.9%)	6 (14.3%)	6.44	0.17
6-10 years	1 (2.6%)	6 (14.3%)		
11-15 years	3 (7.7%)	4 (9.5%)		
16-20 years	5 (12.8%)	1 (2.4%)		
More than 20 years	23 (59.0%)	25 (59.5%)		

Table 4-1 continued.

Demographic Characteristic	Cases (*n* = 66) No. (%)	Controls (*n* = 66) No. (%)	X^2	*p*
Alcohol use	16 (24.2%)	22 (33.3%)	1.33	0.25
Never	50 (75.8%)	44 (66.7%)		
Ever (≥ 1 drink per 6 months)				
Drinks per month [c]	24/46 (47.8%)	18/43 (41.9%)	0.95	0.33
1-19 drinks/month	22/46 (34.4%)	25/43 (58.1%)		
≥ 20 drinks/month				
Frequency [d]	3/49 (6.1%)	0/44 (0%)	4.68	0.32
Once a month	15/49 (30.6%)	10/44 (22.7%)		
2-4 times per month	9/49 (18.4%)	13/44 (29.5%)		
5-8 times per month	8/49 (16.3%)	9/44 (20.5%)		
9-16 times per month	14/49 (28.6%)	12/44 (27.3%)		
≥ 17 times per month				
Use of residential well water (years)				
None	32 (48.5%)	49 (74.2%)	14.19	0.03*
1-3 years	8 (12.1%)	2 (3.0%)		
4-9 years	2 (3.0%)	1 (1.5%)		
10-14 years	5 (7.6%)	2 (3.0%)		
15-20 years	4 (6.1%)	6 (9.1%)		
More than 20 years	12 (18.2%)	6 (9.1%)		
Don't know	3 (4.5%)	0 (0%)		
Years lived on a farm (years)				
None	52 (78.8%)	58 (87.9%)	8.99	0.17
1-3 years	0 (0%)	2 (3.0%)		
4-9 years	4 (6.1%)	2 (3.0%)		
10-14 years	1 (1.5%)	1 (1.5%)		
15-20 years	3 (4.5%)	3 (4.5%)		
More than 20 years	5 (7.6%)	0 (0%)		
Don't know	1 (1.5%)	0 (0%)		

Abbreviations: SD, standard deviation.
Key: Pack years, No. of packs or portion of pack smoked per day multiplied by no. of years smoked.

* = Statistically significant at $p<0.05$

[a] For cases, age at first symptoms was used, and age at interview was used for controls.
[b] A paired t-test was carried out.
[c] Five participants were excluded from analysis due to refusal to disclose the frequency or number of drinks drank per month.
[d] Three participants were excluded from analysis due to refusal to disclose the frequency or number of drinks drank per month.

Table 4-2. Demographic characteristics of age (± 5 years), sex, and race-matched ALS cases and controls.

Table 4-2. Demographic characteristics of age (+ 5 years), sex, and race-matched ALS cases and controls.

Demographic Characteristic	No. cases	No. controls	(Conditional) OR (95% CI)
Education			
High school or less [a]	24	24	1.00 (referent)
More than high school	42	42	1.00 (0.49, 2.05)
Smoking,			
< 1 pack per day	20	20	1.00 (referent)
≥ 1 pack per day	19	22	1.00 (0.32, 3.10)
Alcohol use			
< 17 times per month	35	32	1.00 (referent)
≥ 17 times per month	12	12	0.67 (0.19, 2.36)
Use of residential well water,			
< 15 years	15	5	1.00 (referent)
≥15 years	19	12	0.50 (0.05, 5.51)

Abbreviations: OR, odds ratio; CI, confidence interval.

[a] High school or less included vocational or technical training after high school as well as grade school.
[b] More than high school education level included some college, associate's degree, bachelor's degree or a graduate degree.

Table 4-3. The association of personal risk factors and ALS among matched cases and controls.

Risk Factor	Cases (n = 66) No. (%)	Controls (n = 66) No. (%)	(Conditional) OR (95% CI)
Medical History			
Hypothyroidism, Grave's Disease	2 (3.0%)	4 (6.1%)	0.50 (0.09, 2.73)
Hyperthyroidism	2 (3.0%)	2 (3.0%)	1.00 (0.06, 15.99)
Hyperparathyroidism	0 (0%)	0 (0%)	N/A
Myocardial infarction or coronary thrombosis	7 (10.6%)	4 (6.1%)	2.00 (0.50, 8.00)
Hypertension	30 (45.5%)	27 (40.9%)	1.21 (0.60, 2.46)
Stroke or transient ischemic attack [a]	0 (0%)	2 (3.0%)	0.02 (0.00, 1327.4)
Hyperlipidemia [b]	35 (53.0%)	25/62 (40.3%)	1.73 (0.82, 3.63)
Medication History			
Tranquilizers or muscle relaxants	17 (25.8%)	22 (33.3%)	0.71 (0.34, 1.48)
Psychotherapeutic drugs [c]	6 (9.1%)	6 (9.1%)	1.00 (0.29,3.45)
Cholesterol-lowering drugs	29 (43.9%)	22 (33.3%)	2.00 (0.18, 22.06)
Treatments or Procedures			
Polio immunization [d]	52 (78.8%)	47 (71.2%)	1.60 (0.52, 4.89)
Spinal anesthesia [e]	12 (18.2%)	17 (25.8%)	0.60 (0.22, 1.65)
Trauma/Electrical Shock			
Head injury causing unconsciousness or medical care	16 (24.2%)	19 (28.8%)	0.87 (0.41, 1.82)
Severe injury requiring medical attention	22 (33.3%)	28 (42.4%)	0.63 (0.28, 1.38)
Severe electrical shock	11 (16.7%)	11 (16.7%)	1.00 (0.40, 2.52)
> 1 severe electrical shock [f]	8/11 (72.7%)	7/10 (70.0%)	65.29 (0.00, ∞)
Caffeine and Artificial Sweeteners			
Years (mean, SD)	36.42 ± 18.63	34.09 ± 14.52	4.61 ± 13.68, p=0.045*
Caffeinated coffee			
Diet soda or beverages	17.48 ± 12.80	29.96 ± 12.77	-7.67 ± 20.97, p=0.18
Physical Activity			
Sports [g]	12 (18.2%)	11 (16.7%)	1.11 (0.45, 2.73)
Strenuous activity (median hours per week) [h]			
15-24 years old	5	3	p=0.39
25-34 years old	2	1.5	p=0.94
35-44 years old	2	0	p=0.31
45 years and older	1	0	p=0.54
Military History			
U.S. military service	14 (21.2%)	18 (27.3%)	0.64 (0.25, 1.64)
Males	10/14 (71.4%)	17/18 (94.4%)	N/A
Females	4/14 (28.6%)	1/18 (5.6%)	N/A
Combat	1/14 (7.1%)	4/18 (22.2%)	0.25 (0.03, 1327.44)

Abbreviations: N/A, non-applicable; OR, odds ratio; CI, confidence interval; E, exponential.

* = Statistically significant at $p<0.05$. *P* values for comparisons between cases and controls were calculated using the paired *t* test for continuous variables.

Table 4-3 continued.

[a] One case and one control did not know whether they had experienced a stroke.
[b] One control was unsure if his/her cholesterol level had ever been measured, and four controls had not had their cholesterol levels measured.
[c] One case and one control did not know whether they had taken psychotherapeutic medications.
[d] Six cases and 7 controls did not know if they had received immunization for polio.
[e] Two cases did not know whether they had received spinal anesthesia.
[f] One control did not know whether he/she had received more than one severe shock.
[g] Sports included: intercollegiate sports, amateur competitive athletes, professional or semi-professional athletes.
[h] The Wilcoxon signed rank test was used for non-normally distributed data.

Table 4-4. Family history of neurological conditions among matched ALS cases and controls (n=66 pairs).

	Condition							
Relative	Parkinson's Disease or Parkinsonism	(Conditional) OR (95% CI)	Alzheimer's Disease or dementia	(Conditional) OR (95% CI)	Thyroid Disease	(Conditional) OR (95% CI)	Other diseases affecting nervous system [b]	(Conditional) OR (95% CI)
Mother [c]								
Case	2	2.41 (0.19, ∞) [g]	10	3.33 (0.86, 18.85)	15	1.44 (0.62, 3.38)	3	3.00 (0.24, 157.49) [g]
Control	0		3		10		1	
Father [d]								
Cases	2	2.00 (0.10, 117.99) [g]	4	2.00 (0.29, 22.11) [g]	0	1.00 (0.00. 39.00) [g]	2	2.41 (0.19, ∞) [g]
Control	1		3		1		0	
Siblings [e]								
Case	0	N/A	1	1.00 (0.00, 39.00) [g]	3	0.75 (0.11, 4.43) [g]	2	2.00 (0.01, 117.99) [g]
Control	0		1		4		1	
Children [f]								
Cases	0	N/A	0	N/A	0	N/A	1	1.00 (0.00, 78.50) [g]
Control	0		0		0		1	

Table 4-4 continued.

Abbreviations: N/A, non-applicable; OR, odds ratio; CI, confidence interval.

[a] The total number of pairs included in each analysis is dependent upon participants' knowledge of potential medical conditions of their relatives, or the relative may have been deceased. In some cases, participants did not have siblings or children. In addition, the following conditions: Parkinson's disease, Parkinsonism, Alzheimer's disease, dementia, and thyroid disease did not apply to children under the age of 18.

[b] Other diseases affecting the nervous system included: stroke, epilepsy, multiple sclerosis, spinal disease, traumatic brain injury, cerebrovascular disease, brain tumor, hydrocephalus, central nervous system infections, or others.

[c] 2 cases did not know whether their mother had been diagnosed with Parkinson's Disease or Parkinsonism. 3 cases did not know whether their mother had been diagnosed with Alzheimer's disease or dementia, or with any other disease affecting the nervous system. 2 cases and 1 control did not know whether their mother had been diagnosed with thyroid disease.

[d] 4 cases did not know whether their father had been diagnosed with Parkinson's Disease or Parkinsonism, or with Alzheimer's Disease or dementia. 4 cases and 1 control did not know whether their father had been diagnosed with thyroid disease. 6 cases and 1 control did not know whether their father had been diagnosed with any other disease affecting the nervous system.

[e] 6 cases and 3 controls did not know whether any of their siblings had been diagnosed with Parkinson's Disease or Parkinsonism, or with Alzheimer's Disease or dementia. 6 cases and 6 controls did not know whether any of their siblings had been diagnosed with thyroid disease. 6 cases and 4 controls did not know whether any of their siblings had been diagnosed with any other disease affecting the nervous system.

[f] 12 cases and 13 controls did not know whether their children had been diagnosed with any other disease affecting the nervous system.

[g] Exact conditional logistic regression was performed.

Table 4-5. Risk of ALS according to occupation classification among matched cases and controls

Table 4-5. Risk of ALS according to occupation classification among matched cases and controls.[a]

Occupation	No. of cases (*n* = 66)	No. of controls[b] (*n* = 64)	(Conditional) OR (95% CI)[c]
Managerial and Professional Specialty	14	10	1.38 (0.55, 3.42)
Technical, Sales, and Administrative Support	15	24	0.52 (0.25, 1.09)
Service	9	5	2.00 (0.60, 6.64)
Precision Production, Craft, and Repair	15	19	0.67 (0.27, 1.63)
Operators, Fabricators, and Laborers	10	6	1.67 (0.61, 4.59)
Farming, Forestry, and Fishing	1	0	N/A
Full-time Homemaker	2	0	N/A

Abbreviations: N/A, non-applicable; OR, odds ratio; CI, confidence interval.

[a] Classification of occupation was done according to 1980 Census Industrial and Occupational Classification Codes (Census 1982).

[b] The analysis excluded two controls who did not hold jobs as of the reference date.

[c] The reference group was those not working in the occupational group.

Table 4-6. Risk of ALS according to self-reported occupational exposure among matched cases and controls

Table 4-6. Risk of ALS according to self-reported occupational exposure among matched cases and controls.

Exposure [a]	No. of cases (n = 66)	No. of controls [b] (n = 64)	(Conditional) [c] OR (95% CI)
Metals[1]	19	10	0.89 (0.84, 4.24)
Pesticides[2]	21	8	3.17 (1.27, 7.93)*
Organic/chlorinated solvents[3]	28	31	0.75 (0.38, 1.47)
Aromatic solvents, petroleum and rubber[4]	28	25	1.13 (0.57, 2.27)
Diesel and gasoline fuel[5]	23	18	0.77 (0.34, 1.75)
Antifreeze and coolants	14	10	1.25 (0.49, 3.17)
Electrical/electromagnetic equipment or machinery[6]	33	47	0.41 (0.20, 0.82)

Abbreviations: N/A, non-applicable; OR, odds ratio; CI, confidence interval.

* = Statistically significant at $p<0.05$.

[a] The analysis included occupational exposures occurring 10 or more times throughout occupational history.
[b] The analysis excluded two controls who did not hold jobs as of the reference date.
[c] The reference group included those without the occupational exposure.

[1] Metals included lead and mercury.
[2] Pesticides included: insecticides, herbicides, fungicides, and fumigants.
[3] Organic/chlorinated solvents included: paint strippers, adhesives, degreasers and other cleaning agents, dry cleaning agents, and dyes or printing inks.
[4] Aromatic solvents, petroleum, and rubber included: solvents (such as toluene and xylene), mineral spirits or white spirits, varnishes, oil-based paint, paint thinners, cutting, cooling, and lubrication oils.
[5] Diesel included gasoline and diesel fuel.
[6] Electrical and electronic equipment and machinery included electromagnetic fields such as power lines or transformer stations.

Table 4-7. Risk of ALS and self-reported occupational exposures by lifetime days of exposure among matched cases and controls.

Exposure (days)	No. of cases (*n* = 66)	No. of controls[a] (*n*=64)	(Conditional)[b] OR (95% CI)
Metals[1]			
1-399	2	5	0.45 (0.09-2.34)
400-1,999	5	1	5.0 (0.58-42.79)
≥ 2,000	11	4	2.63 (0.83-8.31)
Pesticides[2]			
1-399	8	4	0.25 (0.05-1.18)
400-1,999	1	2	0.08 (0.08-4.56)
≥ 2,000	8	2	0.01 (0.01-2.18)
Organic/chlorinated solvents[3]			
1-399	4	10	0.37 (0.12-1.21)
400-1,999	6	9	0.64 (0.21-1.94)
≥ 2,000	16	12	1.35 (0.53-3.43)
Aromatic solvents, petroleum and rubber[4]			
1-399	6	7	0.83 (0.28-2.47)
400-1,999	4	6	0.61 (0.14-2.59)
≥ 2,000	14	11	1.39 (0.53-3.67)
Diesel and gasoline fuel [5]			
1-399	2	5	0.43 (0.08-2.24)
400-1,999	6	3	2.46 (0.42-14.26)
≥ 2,000	12	10	1.80 (0.62-5.17)
Antifreeze and coolants			
1-399	4	2	1.52 (0.25-9.28)
400-1,999	2	3	0.33 (0.04-3.21)
≥ 2,000	5	5	1.05 (0.26-4.30)
Electrical and electronic equipment or machinery[6]			
1-399	1	4	0.20 (0.02-1.86)
400-1,999	4	3	1.04 (0.22-4.84)
≥ 2,000	25	39	0.40 (0.19-0.84)

Abbreviations: OR, odds ratio; CI, confidence interval.

[a] The analysis excluded two controls who did not hold jobs as of the reference date.

[b] The reference group was zero lifetime days of exposure to the agent.

[1] Metals included lead and mercury.

[2] Pesticides included: insecticides, herbicides, fungicides, and fumigants.

[3] Organic/chlorinated solvents included: paint strippers, adhesives, degreasers and other cleaning agents, dry cleaning agents, and dyes or printing inks.

[4] Aromatic solvents, petroleum, and rubber included: solvents (such as toluene and xylene), mineral spirits or white spirits, varnishes, oil-based paint, paint thinners, cutting, cooling, and lubrication oils.

[5] Diesel included gasoline and diesel fuel.

[6] Electrical and electronic equipment and machinery included electromagnetic fields such as power lines or transformer stations.

Table 4-8. Conditional logistic regression of the association of personal and occupational risk factors and ALS among matched cases and controls.

Table 4-8. Conditional logistic regression of the association of personal and occupational risk factors and ALS among matched cases and controls.

Variable	All participants[a] (n=130)				95.0% CI	
	B	SE	*p*	Exp(B)	Lower	Upper
Metals	1.295	.590	.028	3.650*	1.149	11.597
Pesticides	1.872	.662	.005	6.500*	1.777	23.772
Organic solvents	.004	.533	.994	1.004	.353	2.856
Aromatic solvents	-.523	.596	.380	.593	.184	1.906
Electrical/Electronic equipment or machinery	-1.291	.467	.006	.275	.110	.686
Education[b]	.214	.468	.648	1.238	.494	3.101
Smoking[c]	-.853	.488	.080	.426	.164	1.108

Abbreviations: OR, odds ratio; CI, confidence interval

Abbreviations: OR, odds ratio; CI, confidence interval; B, beta; SE, standard error; Exp(B), exponentiated beta (odds ratio).

*= Statistically significant at $p<0.05$.

[a] The analysis excluded two controls who did not hold jobs as of the reference date.

[b] Education was categorized as greater than high school or high school or less (referent).

[c] Smoking was categorized as ever (100 or more cigarettes) or never (referent).

*= Statistically significant at $p<0.05$.

[a] The analysis excluded two controls who did not hold jobs as of the reference date.

[b] Education was categoried as great than high school or high school of less (referent).

[c] Smoking was categorized as ever (100 or more cigarettes) or never (referent).

5.0 THE ASSOCIATION OF SUSPECTED NEUROTOXICANT HAZARDOUS AIR POLLUTANTS AND AMYOTROPHIC LATERAL SCLEROSIS AS EVALUATED BY THE NATIONAL-SCALE AIR TOXICS ASSESSMENT DATA

Angela M. Malek, Ph.D.[1]

Aaron Barchowsky, Ph.D. [2]

Robert Bowser, Ph.D. [3]

Terry Heiman-Patterson, M.D. [4]

David Lacomis, M.D. [5]

Sandeep Rana, M.D. [6]

Ada Youk, Ph.D. [7]

Evelyn O. Talbott, Dr.P.H, M.P.H. [1]

[1] Department of Epidemiology, University of Pittsburgh,
Graduate School of Public Health, Pittsburgh, PA 15261

[2] Department of Environmental and Occupational Health, University of Pittsburgh,
Graduate School of Public Health, Pittsburgh, PA 15261

[3] Department of Pathology, University of Pittsburgh,
College of Medicine, Pittsburgh, PA 15261

[4] Department of Neurology, Drexel University, School of Medicine,
School of Medicine, Philadelphia, PA 19107

[5] Department of Neurology, University of Pittsburgh,
School of Medicine, Pittsburgh, PA 15261

[6] Department of Neurology, Temple University School of Medicine,
Philadelphia, PA 19122

[7] Department of Biostatistics, University of Pittsburgh,
Graduate School of Public Health, Pittsburgh, PA 15261

Manuscript in preparation

5.1 ABSTRACT

Background: Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disease in adults. The etiology of ALS is largely unknown; however, a gene-environment interaction is suspected to play a role. The relationship of hazardous air pollutants and ALS has not previously been investigated.

Methods: ALS patients, who were lifelong residents of southwestern Pennsylvania and the greater Philadelphia area, were identified during 2008-2011 from major neurological centers. Residences of age, sex, and race-matched cases and controls were geocoded and linked to the Environmental Protections Agency's National-Scale Air Toxics Assessment data for 1999, 2002, and 2005 to evaluate the relationship of exposure to potentially hazardous air pollutants within census tract of residence and risk of ALS. A total of 33 substances identified in the literature as neurotoxicants were included. Thirty-two substances remained consistent throughout the time period (1999-2005). Odds ratios and 95% confidence intervals were calculated by conditional logistic regression.

Results: Exposure to pesticides was associated with elevated risk of ALS (OR=3.17, 95% CI: 1.27, 7.93) in the 2002 assessment. After adjusting for education and smoking, the final model found an association of other aromatic solvents and increased risk of ALS in 1999 (OR=14.75, 95% CI: 1.05, 209.98). Exposure to pesticides was also related to elevated risk of ALS in 1999 (OR=3.52, 95% CI: 1.05, 11.76). Possible explanations for the differing concentrations of substances over time include real changes in emissions or source characterization as well as methodology advancements.

Conclusion: A potential association is suggested by increased ambient air concentration of hazardous air pollutants, especially pesticides and solvents, among place of residence and risk of

ALS. The public health significance of this research includes knowledge gained in a new area of ALS research examining exposure to hazardous air pollutants. Future research should further examine the effects of hazardous air pollutants at the residential level as well as at an occupational level.

Key words: ALS, NATA, environmental exposures, hazardous air pollutants, neurotoxicants.

5.2 INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disease (MND) in adults with an annual incidence of 1-3 per 100,000 persons worldwide (Doi, Kikuchi et al. 2006; Migliore and Coppede 2009). The average age of onset is 58-63 years. In general, ALS increases with age until age 75 (Migliore and Coppede 2009). ALS occurs more often among men than women with a male to females ratio of 3:2; however, the incidence balances out at menopause (Kamel, Umbach et al. 2005; Migliore and Coppede 2009). Median survival of ALS is about 2-4 years following disease onset (Borasio and Miller 2001).

There are very few known risk factors for ALS identified from previous epidemiologic investigations, and those identified are very general and include male sex and age (Nelson 1995; Morahan and Pamphlett 2006). The 3:2 male to female ratio argues for a possible environmental or occupational exposure not experienced in a widespread manner in women. Genetic susceptibility to various environmental exposures is also suspected to be related to ALS. Moreover, the relationship of hazardous air pollutants and ALS has not previously been investigated.

Several neurotoxicants have been linked to neurological conditions such as: solvents (toluene), pesticides (organochlorines and organophosphates), ethyl alcohol, polychlorinated biphenyl compounds (PCBs), and heavy metals (methylmercury, arsenic, manganese, and most notably lead) (Nelson 2004; Miodovnik 2011). Other suspected neurotoxicants include: trichloroethylene (TCE), tributyltin, cadmium, perflurochemicals (PFCs), bisphenol A (BPA), polybrominated diphenyl ethers (PBDEs), dioxins (polychlorinated dibenzodioxins (PCDDs) and dibensofurans (PDBFs), acrylamide, and polycyclic aromatic hydrocarbons (PAHs) (Miodovnik 2011).

At least 16 studies have found an association between lead and risk of ALS (Campbell, Williams et al. 1970; Felmus, Patten et al. 1976; Rosati, Pinna et al. 1977; Schwarz 1977; Conradi, Ronnevi et al. 1978; Roelofs-Iverson, Mulder et al. 1984; Gresham, Molgaard et al. 1986; Armon, Kurland et al. 1991; Gunnarsson, Bodin et al. 1992; Chancellor, Slattery et al. 1993; Strickland, Smith et al. 1996; Vinceti, Guidetti et al. 1996; McGuire, Longstreth et al. 1997; Longnecker, Kamel et al. 2000; Kamel, Umbach et al. 2002; Armon 2004). However, several studies have failed to find an association for exposure to lead and risk of ALS (Gresham, Molgaard et al. 1986; McGuire, Longstreth et al. 1997; Vinceti, Guidetti et al. 1997; Gait, Maginnis et al. 2003).

Exposure to other metals such as selenium and mercury has also been investigated in relation to ALS risk. Selenium was found to be related to ALS by three studies (Kilness and Hichberg 1977; Vinceti, Guidetti et al. 1996; Vinceti, Bonvicini et al. 2010). Exposure to mercury was related to the potential for ALS development by one study (Provinciali and Giovagnoli 1990). In some cases, the association of metal exposure and ALS was not replicated (Gait, Maginnis et al. 2003) and the specific metals: mercury, aluminum, cadmium, chromium, and manganese were not found to be associated with ALS (McGuire, Longstreth et al. 1997).

The association of exposure to solvents and risk of ALS has produced inconsistent findings. Several studies have reported a relationship between solvents (i.e., cleaning solvents and degreasers, polychlorinated biphenyls (PCBs), hairdresser and cosmetology occupations) and risk of ALS (McGuire, Longstreth et al. 1997; Park, Schulte et al. 2005; Steenland, Hein et al. 2006), while others investigating alcohols or ketones, benzene, styrene, phenols, paints, solvent-based inks or dyes, and adhesives have failed to produce an association (Welp, Kogevinas et al. 1996; McGuire, Longstreth et al. 1997; Gait, Maginnis et al. 2003; Park, Schulte et al. 2005).

Few studies have been conducted to date to evaluate the relation of exposure to specific solvents and risk of ALS.

Results have also been inconsistent for the association of exposure to pesticides and risk of ALS. An association has been confirmed by some studies (McGuire, Longstreth et al. 1997; Burns, Beard et al. 2001; Park, Schulte et al. 2005) and refuted for exposure to different pesticide classes or concentrations by two of the same studies (McGuire, Longstreth et al. 1997; Burns, Beard et al. 2001).

Although a number of studies have been conducted to examine the potential neurotoxic effects of heavy metal exposure and risk of ALS, many solvents have yet to be investigated. Existing data for known neurotoxicants in ambient air pollution is provided through the Environmental Protection Agency's (EPA) National-Scale Air Toxics Assessment (NATA) data for four years: 1996, 1999, 2002, and 2005. This presents an opportunity to examine the association of neurotoxicants in relation to residence and development of ALS.

NATA uses a national air dispersion model based on emissions and monitoring stations including toxic release inventories (TRI) and mobile and stationary emissions sources such as point, non-point, and mobile (both on and off-road) sources on both a county and census tract grid. Point sources include factories and large waste incinerators while non-point sources refer to small manufacturing facilities, gas stations, and dry cleaners (EPA 2011). Cars and trucks comprise on-road mobile sources (defined as vehicles found on highways), and off-road sources include trains and ships (EPA 2011). Also included in the model are background sources which involve previously emitted anthropogenic air toxics and natural sources that may remain in the environment, as well as emitted air toxics of distant sources transported more than 50 kilometers away. Although not created for exposure assessment investigations, NATA does purport to

show areas with greater industrial activity and the presence of byproducts of combustion and chemical use.

The potential association of suspected neurotoxicants found in air pollution is an interesting one to explore, especially given the urban environments of Pittsburgh and Philadelphia and the nearby industries. To assess the potential role of ambient air pollution and the development of ALS, the case-control analysis described in this paper links residential data of cases and controls to NATA data for hazardous air pollutants (HAPs).

5.3 METHODS

5.3.1 Study Population

The study design has been previously described in detail elsewhere (unpublished). This study received approval by the University of Pittsburgh Institutional Review Board, Allegheny General Hospital Institutional Review Board, and Drexel University College of Medicine Institutional Review Board. All participants provided written informed consent. The specific aim was to investigate the association of suspected neurotoxicants found in ambient air pollution and the risk of ALS.

Sporadic ALS cases were recruited between 2008 and 2011, over a 24-month period, from three neurology clinics with ALS centers; two in Pittsburgh, PA and one in Philadelphia, PA. The World Federation of Neurology El Escorial criteria were used by board certified neurologists for ALS diagnosis (Brooks 1994). Patients with possible, probable, and definite ALS were included in the study. The study area consisted of 6 W. Pa counties (Allegheny,

Armstrong, Beaver, Butler, Washington, and Westmoreland), and 6 greater Philadelphia area counties (New Castle, Delaware (DE); Union County, New Jersey (NJ); Somerset, NJ; Sussex, NJ; Montgomery, PA; and Philadelphia, PA). Cases and controls were required to live in one of the above study counties for at least one year prior to the study.

Controls were matched to cases by 1:1 matching on age of case's first ALS symptoms ($\pm$ 5 years), sex, race, and region (W. Pa or the greater Philadelphia area) and included both outpatient hospital controls and population controls. The response rate for eligible controls was 70% for W. Pa outpatient hospital controls and 4.6 % for the greater Philadelphia area InfoUSA mailing. The average response rate for an InfoUSA mailing is 2%-3% (Infogroup 2011).

5.3.2 Exposure Assessment

A modified version of the ALS Consortium of Epidemiologic Studies (ACES) ALS risk factor questionnaire (ALSRFQ) was administered by personal interview to obtain information on lifetime residential and occupational history, vocation and avocation exposures, and personal lifestyle factors (ACES 2005). This questionnaire was used by McGuire et al.'s 1997 study as well as by others (McGuire, Longstreth et al. 1997). Occupations of participants were characterized by six occupational categories according to the 1980 U.S. Census Industrial and Occupational Classification Coding System which included: 1) managerial and professional specialty; 2) technical, sales, and administrative support; 3) service; 4) precision production, craft, and repair; 5) operators, fabricators, and laborers; and 6) farming, forestry, and fishing (Census 1982). Full-time homemakers were included in a seventh category.

Residential addresses of cases and controls were geocoded and linked to EPA NATA data for HAPs by census tract for the years 1999, 2002, and 2005 using GeocodeDVD and ESRI's

ArcGIS StreetMap North America (version 10; ESRI Inc., Redlands, CA) (ESRI 2010; GeoLytics 2011). States of residence for participants during the three years of NATA included: Ohio, Delaware, California, Florida, with the majority living in Pennsylvania. If a participant had more than one address during a particular NATA assessment year, the first address was used as it was assumed this would capture any exposure occurring prior to the NATA measurement. Batch processing was used to successfully geocode 91% (n=84) of residences with complete addresses for 1999, 95% (n=108) for 2002, and 93% (n=98) for 2005. The number of addresses successfully geocoded improved over time; however, several case/control addresses were unable to be geocoded for 1999, 2002 and 2005 (49, 37, and 35, respectively) due either to recall bias or the inability of addresses to be successfully geocoded by ArcMAP, and thus were excluded from analysis.

NATA data is available for the years: 1996, 1999, 2002, and 2005, by census tract resolution. Estimated inhalation exposure concentrations of outdoor emissions and diesel particulate matter (PM) (referred to as air toxics) are used to describe potential non-cancer effects and risk of cancer (EPA 2011). This paper evaluated results from the three most recent NATA assessments (1999, 2002, and 2005). Air toxic emissions have declined 40% since the Clean Air Act Amendment was passed in 1990 (EPA 2011). As a result of improvements in air quality conditions over time along with methodological changes in NATA assessments, concentrations of substances being evaluated and the associated health outcomes vary by time point. Advancements have been made in NATA assessments over time such as: improvements in point and non-point source characterization, background concentration characterization, risk characterization, and National Emission Inventory (NEI) inventory, as well as updated exposure approaches (EPA 2011).

The number of air toxics included for evaluation varied for each year of NATA. Of the 187 air toxics identified by the Clean Air Act, 177 toxics as well as diesel PM, were evaluated by NATA in the 1999 and 2005 assessments (EPA 2011). In 2002, a total of 180 air toxics were evaluated in addition to diesel PM. Cancer and non-cancer results are available for all substances in which chronic exposure-based health data exist except for diesel PM, which only included non-cancer results. Fifty-seven air toxics lacked information on health effects in the 2002 assessment, and 39 lacked information in 2005. The 2005 NATA assessment also excluded 10 air toxics with unreliable emission estimates (e.g., radionuclides) and those with missing emissions information (EPA 2011).

Suspected neurologic toxicants and chemicals of concern were assessed in our study (ATSDR 2000; CalEPA 2005). This included a total of 33 suspected neurotoxicants grouped structurally by: 1) metals, 2) aromatic solvents, 3) organic/chlorinated solvents, 4) other HAPs, and 5) pesticides. Concentrations of the various substances evaluated had different orders of magnitude and therefore could not be combined by groups. Instead, quartiles were used to help normalize the data and an index score was calculated for each group. Each compound was assigned a rank of one (low) to four (high) based on the associated quartile. Then, an average of the quartiles for all compounds in a group (i.e., metals, organic/chlorinated solvents, aromatic solvents, and pesticides) was taken. Finally, this overall group average was categorized into quartiles.

Compounds with an exposure concentration of zero and compounds unavailable through NATA for a particular census tract or year were not factored into the average of the overall group quartiles. In addition, certain compounds were excluded from the group quartile

estimation for years in which very little variability was observed among cases/controls. The above process was repeated for the three years of NATA data (1999, 2002, and 2005).

5.3.3 Statistical Analysis

Univariate analyses were carried out to explore demographic characteristics of cases and controls. Cases' and controls' mean concentrations of exposure to the various chemical compounds were assessed by paired t-tests. Odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were calculated by conditional logistic regression to explore the relationship of exposure to groups of hazardous air pollutants by the upper quartiles (3^{rd} and 4^{th}) and risk of ALS. Quartiles one and two were the referent group. Separate models were calculated for each group of compounds. A final model was also created. Two-tailed tests were used and statistical significance involved p-values<0.05. IBM SPSS (Statistical Package for the Social Sciences) Statistics, version 19, was used to conduct all analyses (IBM SPSS 2010).

5.4 RESULTS

Demographic characteristics of cases and controls from the original study are displayed in Table 1. The majority of cases and controls were male (68.2%) and Caucasian or White race (98.5%). Cases were somewhat older than controls (57.12 $\pm$ 13.23 years vs. 56.38 $\pm$ 13.49 years, respectively) (paired t-test, p=0.07). The large majority of cases and controls were less than 75 years of age; 9.1% of cases and 6/1% of controls were 75 years or older. Approximately 30% of both cases and controls had obtained a high school degree. The majority of cases (54.6%) and

controls (59.1%) had attended some college or had obtained an associates, bachelors, or graduate degree. Only 9.1% of cases and controls were single and had never been married. Over 80% of cases and 75.8% of controls were married or cohabiting. Number of years lived at the residence for each year of NATA was assessed. On average, cases lived at the residence longer than controls although this difference was not statistically significant. In 1999, cases reported living in their residence 20.03 years (standard deviation, 13.13) vs. 17.39 years for controls (12.30). In 2002, cases lived in the residence for 20.49 years (15.67) compared to 17.93 (12.60) for controls. A similar pattern was seen in 2005 with cases living in the residence for 23.78 years (16.60) vs. controls who lived in their residence for an average of 20.60 years (14.45).

Occupation is also shown in the table. Due to the small number of cases and controls in each category, the 1980 U.S. Census occupation coding was used. There were no significant differences among any of the occupational categories for cases and controls. Roughly one fourth of cases were employed in skilled jobs (precision, production, craft and repair) (22.7%), approximately one fourth were in professional positions (21.2%), and 15.2% were in unskilled jobs (operators, fabricators, and laborers), compared to controls (29.7%, 15.6%, and 9.4%, respectively). In addition, one fourth of cases worked in technical, sales, or administrative positions (22.7%) and 13.6% worked in service jobs compared to the controls (37.5% and 7.8%, respectively). One case was employed in farming, forestry, or fishing and two cases were full-time homemakers; no controls worked in these positions. More male cases than controls worked in unskilled jobs (8 vs. 5), while fewer male cases worked in skilled jobs compared to controls (13 vs. 9). More male cases were employed in professional positions than controls (11 vs. 6).

Slightly fewer cases (59.1%) than controls (63.6%) had ever smoked cigarettes (defined as smoking at least 100 cigarettes). Cases were more likely than controls to drink alcohol once a

month (6.1% vs. 0%), 2-4 times per week (30.6% vs. 22.7%), and 17 or more times per month (28.6% vs. 27.3%). More controls drank alcohol 5-8 times per month (29.5% vs. 18.4%) and 9-16 times per month (16.3% vs. 20.5%) compared to cases.

5.4.1 Demographic Characteristics of Age-Stratified Cases and Controls

Table 2 displays demographic characteristics of cases and controls stratified by age. Twenty-five cases and 30 controls were between the ages of 18-54 with a mean age of 43.56 $\pm$ 8.84 (standard deviation, SD) and 44.43 $\pm$ 8.88, respectively. The mean age and SD of the 31 cases and 36 controls aged 55 or older was 65.39 $\pm$ 7.25 and 66.33 $\pm$ 6.97, respectively. In both age groups, cases were slightly younger than controls. The proportion of males and females was similar for cases and controls although varied by age group with about 75% males among those 18-54 years of age and about 64% males of those 55 and older. Females made up approximately 25% of the younger group and 36% of the older age group. The majority of participants were white; however, one pair in the 55 and older group was African-American.

Education was mostly similar among those 18-54 years of age but a significant difference was seen among those 55 and older. More controls than cases received some college education, an associates degree, or a graduate degree. Marital status was similar for cases and controls in both age groups. There were no significant differences for occupation of cases and controls among those aged 18-54 years or those 55 years of age or older.

Smoking and alcohol use were also assessed. Controls in the younger age group were more likely to have ever smoked cigarettes compared to cases (defined as smoking 100 or more cigarettes) (73.3% vs. 56%). In the older age group, slightly more cases than controls reported ever smoking (61% vs. 55.6%). Frequency of alcohol drank per month was similar among cases

and controls in both age groups. Among those aged 18-54 years, cases were more likely to drink alcohol 2-4 times per month, 9-16 times per month, or 17 or more times per month compared to controls although there was not a significant difference. A larger proportion of cases aged 55 and older drank alcohol once per month or 2-4 times per month than did controls. Controls in the older age group were more likely than cases to drink alcohol 5-8 or 9-16 times per month.

5.4.2 Hazardous Air Pollutants by Individual Chemical Compound

The association of concentrations of hazardous air pollutants [expressed in micrograms per cubic meter of air ($\mu g/m^3$)] and risk of ALS among matched cases and controls in W. Pa and the greater Philadelphia area (as well as for the 1999 (n=37 pairs), 2002 (n=45 pairs), and 2005 (n=45 pairs) NATA assessments is presented in Table 3. Also included were a few participants living in a state other than Pa during NATA assessment years (Ohio, California, Delaware, or Florida). Individual chemical compounds are categorized by exposure group and displayed by means, standard deviations, and paired t-test results. No significant differences were found for cases or controls for the first group, metals (n=7), for the three years. Metals included: arsenic, cadmium, lead, manganese, mercury, nickel, and selenium.

The next group consisted of aromatic solvents (n=6), which may be used as mineral spirits or white spirits; varnishes; oil-based paint; paint thinners; or cutting, cooling, and lubricating oils. In 1999, no significant differences were found. In 2002, 2,4,dinitrotoluene concentrations were significantly greater for cases compared to controls (p=0.08), as were concentrations of benzene (p=0.045), ethyl benzene (p=0.02), styrene (p=0.01), toluene (p=0.02), and xylene (p=0.03). Styrene concentrations remained elevated in 2005 although the difference was borderline

significant (p=0.07). No other significant differences in aromatic solvent concentrations were found among cases and controls during 2005.

Benzene is produced by the burning of several natural products. It is found in gasoline and others fuels. The manufacture of detergents, plastics, pesticides and other chemicals involves benzene. Benzene is a carcinogen that may cause leukemia and blood and bone marrow defects. Neurological symptoms can result from short-term exposure. Those most at risk of occupational exposure to benzene are employee of the construction industry, shipyard, or other industries.

Ethyl benzene is used in the manufacture of paints, varnishes, surface coatings, styrene monomer, cellulose acetate, synthetic rubber, and rubber adhesive and its application. Workers involved in these manufacturing industries may be at risk of ethyl benzene exposure. Symptoms of exposure include skin, eye, and mucous membrane irritation, as well as other mild neurological effects (headache, incoordination, etc.).

Styrene has many uses. It is used as a feedstock for synthetic rubber as well as in the production of many products such as carpet backing, boat parts, food containers, insulation, and piping, that are made from the following substances: polystyrene plastic, fiberglass reinforced plastics, styrenated polyester, resins, and protective coatings. Styrene poisoning can affect the eyes, nose, throat, skin, and lungs causing symptoms like nausea, headaches, dizziness, drowsiness, and clumsiness. It can also damage chromosomes. Greater exposure may occur among workers involved with polymerization, styrene-butadiene rubber production, in the fiberglass and reinforced plastics industries, as well as among boat builders and repairers.

Toluene is used to make benzene, xylene, polyurethane flexible foams (upholstery, mattresses and automotive seats), and can be used as a solvent. It is also used in gasoline as an octane booster. Production of paint and paint thinners, adhesives, lacquers, fingernail polish,

rubber, and some printing and leather tanning processes may also involve toluene. Exposure to toluene on the job may occur among those working with paints, lacquers, gasoline, kerosene, heating oil, and as photogravure printers. Symptoms of toluene exposure include: headaches, tiredness, weakness, confusion, memory loss, nausea, redness, and loss of appetite. Hearing and vision color loss are long-term effects.

Xylene is used as a diluent and solvent, in the formulation on insecticides, dry ice, as a feedstock, and in the production of many other products such as pharmaceuticals, dyes, vitamins, leathers, quartz crystal oscillators, hydrogen peroxide, and perfume. On the job exposure may involve individuals working in printing, rubber, or leather industries as well as in hydraulic fracking. As a result of xylene exposure, reaction time, balance, and memory may be affected as well as eye irritation. Dopamine, noradrenaline and glutathione can also be affected.

Organic/chlorinated solvents (n=13) comprised the largest group of exposures and included substances used as paint strippers, adhesives, degreasers and other cleaning agents, dry cleaning agents, and dyes or printing inks. No differences were found for concentrations of organic/chlorinated solvents among cases compared to controls in 1999. In 2002, hexane concentrations were significantly elevated in cases compared to controls (p=0.01). Also elevated and borderline significant for cases compared to controls in 2002 were concentrations of methyl chloride (p=.096). Controls had borderline significantly lower concentrations of methyl chloride than cases in 2005 (p=0.08). Cases and controls did not differ significantly in their estimated concentrations of any of the other organic/chlorinated solvents [1,1,2,2- tetrachloroethane, 1,1,1-trichloroethane (methyl chloroform), tetrachloroethylene, carbon disulfide, carbon tetrachloride, chloroform, cresols and cresylic acid, ethylene oxide, methylene chloride, trichloroethylene, or vinyl chloride] for any of the three years.

Hexanes are used in gasoline, textile manufacturing, for cleansing and degreasing, as solvents, and as glues for roofing, leather products, and shoes. Short-term effects of hexane include headaches, nausea, drowsiness, and mild euphoria. Long-term exposure can cause symptoms such as tingling and cramping in the arms and legs, coordination loss, vision problems, general muscular weakness, muscle atrophy, and failure of the peripheral nervous system. Factory workers involved in mixing and drying jobs, printing, working with tungsten carbide alloys, or those working at petrochemical plants may be exposed to hexanes.

Methyl chloride occurs naturally in the environment; however, it is also used in silicone, agricultural chemicals, methyl cellulose, quaternary amines, and butyl rubber production, as well as for other uses. Occupational exposure may occur during production of these compounds. Methyl chloride was previously used as a refrigerant but has since been replaced by Freon and other substances. Exposure to methyl chloride can cause serious nervous system effects such as vision and eye problems, tremors, dizziness, memory loss, or even a coma.

The other HAPs group included six chemical compounds. There were no significant differences in concentrations of other HAPs [allyl chloride, cyanide, hexachloroethane, hydrazine, or polychlorinated biphenyls (PCBs)] among cases and controls for the three years of NATA. Allyl chloride estimates were not available in 2002 for our sample.

The last group consisted of pesticides (n=3). Concentrations of ethylene dibromide, ethylene dichloride, and hexachlorobenzene were not significantly different among cases and controls for any of the years. Hexachlorobenzene was not available for 2005.

5.4.3 Hazardous Air Pollutants by Groups of Chemical Compounds

Table 4 displays results for conditional logistic regression of the 3rd and 4th quartiles (above the median) of groups of chemical compound for 1999 (n=36 pairs), 2002 (n=45 pairs), and 2005 (n=45 pairs). The 1st and 2nd quartiles served as the reference group. Chemical compounds with very little variability for the included residential census tracts were excluded from the group analysis. Metals included: arsenic, cadmium, lead, manganese, mercury, nickel, and selenium. Aromatic solvents consisted of: 2,4-dinitrotoluene, benzene, ethyl benzene, styrene, toluene, and xylene. Organic/chlorinated solvents were comprised of: 1,1,1-trichloroethane (methyl chloroform), 1,1,2,2-tetrachloroethane, carbon disulfide, carbon tetrachloride, chloroform, cresols and cresylic acid, ethylene oxide, hexane, methyl chloride, methylene chloride, tetrachloroethylene (perchloroethylene), trichloroethylene, and vinyl chloride. Other HAPs included: acrylamide, allyl chloride, cyanide compounds, hexachloroethane, hydrazine, and polychlorinated biphenyls (PCBs). The final group, pesticides, consisted of ethylene dibromide, ethylene dichloride, and, hexachlorobenzene.

In 2002, an association was found for exposure to high concentrations (3^{rd} and 4^{th} quartiles) of pesticides and elevated risk of ALS (OR=3.17, 95% CI: 1.27, 7.93). The 1^{st} and 2^{nd} quartiles served as the reference group. There were no significant differences for metals, organic/chlorinated solvents, aromatic solvents, or other HAPs for the three assessment periods.

5.4.4 Final Model

A final conditional logistic regression model was constructed for each of the three years of NATA data to compute the estimated odds ratio of developing ALS among persons who had

lived in a census tract with greater exposure (3rd and 4th quartiles) to suspected neurotoxicant air pollutants by structurally similar groups of compounds (metals, aromatic solvents, organic solvents, and other haps) relative to persons who lived in a census tract with lesser exposure to the pollutants (1st and 2nd quartiles). The final models are displayed by year in Tables 5, 6, and 7. The matched study design controlled for age (± 5 years), sex, and race. The groups of compounds have been previously defined.

An association of other aromatic solvents and increased risk of ALS in 1999 (OR=14.75, 95% CI: 1.05, 209.98) after adjusting for education (defined as greater than high school vs. high school or less) and smoking (ever vs. never). Exposure to pesticides was also related to elevated risk of ALS in 1999 (OR=3.52, 95% CI: 1.05, 11.76) after adjusting for education and smoking. In 1999 and 2005, risk of ALS did not differ significantly from those without exposure, after adjusting for education and smoking.

5.5 DISCUSSION

A potential association is suggested by elevated ambient air concentrations of hazardous pollutants among place of residence and risk of ALS. This study is the first to evaluate the association of air pollutants and ALS. Our study found a relationship between exposure to ambient air pollutants, specifically pesticides and solvents, and risk of ALS. Heavy metals, pesticides, and other environmental and occupational exposures have been investigated as potential risk factors for ALS although findings have been inconsistent. Although not causally related, lead has been the most consistent environmental risk factor over time; however, this finding was not replicated by our study.

This study is the second of two investigating the relationship of environmental, occupational, and personal risk factors for ALS among W. Pa and greater Philadelphia area residents. As other studies have lacked additional sources of individual-level exposure such as smoking, occupation, avocation, etc., this is a major strength of our study. A lifetime occupational history for participants was obtained that included self-reported occupational exposures. Results from our first study found an association of occupational exposure to metals and increased risk of ALS after controlling for smoking and education (OR=3.65, 95% CI: 1.15, 11.60, p=0.03) (unpublished). Occupational exposure to pesticides was also related to elevated ALS risk after controlling for smoking and education (OR=6.50, 95% CI: 1.78, 23.77, p=0.005) (unpublished). However, we did not find a relationship between occupational exposure to organic solvents, aromatic solvents, or electrical and electronic equipment or machinery and ALS (unpublished).

Our current study had several limitations. Chemical compounds were combined into structurally related groups using an index score to attempt to normalize the data that varied by orders of magnitude. The index score calculation method was based on the work of others and was modified slightly (Windham, Zhang et al. 2006). Although concentrations of chemical compounds were not available on an individual level, these ambient air estimates are believed to be valid measures of exposure (Payne-Sturges, Burke et al. 2004). The place of residence may have changed during the three assessment periods for some participants; however, we found that the majority of participants remained in the same residence. To account for those who had lived in more than one address during a NATA assessment year, the first residence was included in the analysis to attempt to capture exposure occurring prior to the assessment period. Although significant associations were found for ambient air concentrations of pollutants and ALS, our

power and sample size may have been improved with complete residential history ascertainment as this would have increased the percentage of geocoded residences.

Several hazardous ambient air pollutants were associated with ALS by place of residence in univariate paired analysis (aromatic solvents and organic/chlorinated solvents in 2002 and 2005) as well as in multivariate analysis of the 3rd and 4th quartiles of structurally similar exposure groups (pesticides in 2002) compared to the 1st and 2nd quartiles. The final model found a relationship between greater exposure (3rd and 4th quartiles) to concentrations of other aromatic solvents and pesticides and increased risk of ALS for the 1999 NATA assessment after adjusting for smoking and education. No other groups of compounds were associated with increased risk of ALS for the 1999, 2002, or 2005 NATA assessments.

Possible explanations for differing findings by year of NATA assessment may include real changes in emissions or source characterization, advancements in methodology, or equipment used. Changes in climate, weather, industries, automobile fuel combustion, and power generation are also possible explanations. The associations of exposure to pesticides and aromatic solvents and risk of ALS were found for the year 1999 while controlling for education and smoking. As education and smoking are related to exposure it appears as though the increases in concentration of compounds may have been real. More environmental exposures may have occurred in 1999 than in the two more recent NATA assessment years (2002 and 2005). This finding is important as 1999 may be a crucial time point for the vast majority of ALS patients in our study who had not yet developed symptoms or been diagnosed with the disease. The trigger for ALS is unknown; however, exposures from ten years past may provide a clue as to the etiology of disease. Future ALS research should continue to further examine the effects of hazardous air pollutants at the residential level as well as at an occupational level.

5.6 TABLES

Table 5-1. Demographic characteristics of ALS cases and controls.[a]

Demographic Characteristic	Cases (*n* = 66) No. (%)	Controls (*n* = 66) No. (%)	*p*
Sex			
Male	45 (68.2%)	45 (68.2)	1.00
Female	21 (31.2%)	21 (31.2%)	
Race			
Caucasian or White	65 (98.5%)	65 (98.5%)	1.00
African-American or Black	1 (1.5%)	1 (1.5%)	
Age (years)			
Mean ± SD [b]	57.12 ± 13.23	56.38 ± 13.49	0.07**
18-44	12 (18.2%)	13 (19.7%)	0.87
45-54	13 (19.7%)	17 (25.8%)	
55-64	17 (25.8%)	17 (25.8%)	
65-74	18 (27.3%)	15 (22.3%)	
≥ 75	6 (9.1%)	4 (6.1%)	
Years at residence (mean ± SD) [b]			
1999 NATA assessment (n=72)	20.03 ± 13.13	17.39 ± 12.30	0.35
2002 NATA assessment (n=90)	20.49 ± 15.67	17.93 ± 12.60	0.40
2005 NATA assessment (n=90)	23.78 ± 16.60	20.60 ± 14.45	0.33
Education			
Grade school (1-8 years)	4 (6.1%)	2 (3.0%)	0.70
High school (9-12 years)	20 (30.3%)	22 (33.3%)	
Vocational/technical training	6 (9.1%)	3 (4.5%)	
Some college	17 (25.8%)	17 (25.7%)	
Associates degree	2 (3.0%)	6 (9.1%)	
Bachelors degree	11 (16.7%)	10 (15.2%)	
Graduate degree	6 (9.1%)	6 (9.1%)	
Marital status			
Single and Never Married	6 (9.1%)	6 (9.1%)	0.56
Married or Cohabiting	54 (81.8%)	50 (75.8%)	
Divorced or Widowed	6 (9.1%)	10 (15.2%)	
Occupation [c d]			
Managerial and Professional Specialty	14 (21.2%)	10 (15.6%)	0.22
Technical, Sales, and Administrative Support	15 (22.7%)	24 (37.5%)	
Service	9 (13.6%)	5 (7.8%)	
Precision Production, Craft, and Repair	15 (22.7%)	19 (29.7%)	
Operators, Fabricators, and Laborers	10 (15.2%)	6 (9.4%)	
Farming, Forestry, and Fishing	1 (1.5%)	0 (0%)	
Full-time Homemaker	2 (3.0%)	0 (0%)	
Smoking status			
Never	27 (40.9%)	24 (36.4%)	0.59
Ever (≥ 100 cigarettes)	39 (59.1%)	42 (63.6%)	
Alcohol use (Frequency) [e]			
Once a month	3/49 (6.1%)	0/44 (0%)	0.32
2-4 times per month	15/49 (30.6%)	10/44 (22.7%)	
5-8 times per month	9/49 (18.4%)	13/44 (29.5%)	
9-16 times per month	8/49 (16.3%)	9/44 (20.5%)	
≥ 17 times per month	14/49 (28.6%)	12/44 (27.3%)	

Abbreviations: No., number; SD, standard deviation.

* = Statistically significant at $p<0.05$. **= Statistically significant at $p<0.10$.

Table 5-1 continued.

[a] Data is provided for the original study sample although the number of participants varied for the three comparison years as a result of the number of successfully geocoded residences.
[b] Paired t-test was carried out for mean age and mean years living at residence as of each NATA assessment.
[c] Occupation classified according to 1980 Census Industrial and Occupational Classification Codes .
[d] The analysis excluded two controls who did not hold jobs as of the reference date.
[e] The analysis included 49 cases and 44 controls who reported drinking alcohol once a month or more.

Table 5-2. Demographic characteristics of ALS cases and controls stratified by age.[a]

Demographic Characteristic	18-54 years: Cases (*n* = 25) No. (%)	18-54 years: Controls (*n* = 30) No. (%)	*p*	55 years and greater: Cases (*n* = 31) No. (%)	55 years and greater: Controls (*n* = 36) No. (%)	*p*
Sex						
Male	19 (76%)	22 (73.3%)	0.82	26 (63.4%)	23 (63.9%)	0.97
Female	6 (24%)	8 (26.7%)		15 (36.6%)	13 (36.1%)	
Race						
Caucasian or White	25 (100%)	30 (100%)	N/A	40 (97.6%)	35 (97.2%)	0.93
African-American or Black	0 (0%)	0 (0%)		1 (2.4%)	1 (1.3%)	
Age (years)						
Mean ± SD	43.56 ± 8.84	44.43 ± 8.88	0.72	65.39 ± 7.25	66.33 ± 6.97	0.56
Education						
Grade school (1-8 yrs.)	0 (0%)	2 (6.7%)	0.64	4 (9.8%)	0 (0%)	0.06
High school (9-12 yrs.)	7 (32%)	11 (36.7%)		12 (29.3%)	11 (30.6%)	**
Vocational/technical training	1 (4%)	2 (6.7%)		5 (12.2%)	1 (2.8%)	
Some college	8 (32%)	5 (16.7%)		9 (22%)	12 (33.3%)	
Associates degree	2 (8%)	2 (6.7%)		0 (0%)	4 (11.1%)	
Bachelors degree	3 (12%)	6 (20%)		8 (19.5%)	4 (11.1%)	
Graduate degree	3 (12%)	2 (6.7%)		3 (7.3%)	4 (11.1%)	
Marital status						
Single and Never Married	5 (20%)	6 (20%)	0.81	1 (2.4%)	0 (0%)	0.44
Married or Cohabiting	18 (72%)	20 (66.7%)		36 (87.8%)	30 (83.3%)	
Divorced or Widowed	2 (8%)	4 (13.3%)		4 (9.8%)	6 (7.8%)	
Occupation [b,c]						
Managerial and Professional Specialty	7 (28%)	3 (10.3%)	0.34	7 (17.1%)	7 (20%)	0.66
Technical, Sales, and Admin. Support	6 (24%)	14 (48.3%)		9 (22%)	6 (17.1%)	
Service	3 (12%)	3 (10.3%)		4 (9.8%)	6 (17.1%)	
Precision Production, Craft, and Repair	5 (20%)	6 (20.7%)		10 (24.4%)	11 (31.4%)	
Operators, Fabricators, and Laborers	2 (8%)	3 (10.3%)		1 (2.4%)	5 (14.3%)	
Farming, Forestry, and Fishing	1 (4%)	0 (0%)		0 (0%)	0 (0%)	
Full-time homemaker	1 (4%)	0 (0%)		1 (2.4%)	0 (0%)	
Smoking status						
Never	11 (44%)	8 (26.7%)	0.18	16 (39%)	16 (44.4%)	0.63
Ever (≥ 100 cigarettes)	14 (56%)	22 (73.3%)		25 (61%)	20 (55.6%)	
Alcohol use (frequency) [d]						
Once per month	0/20 (0%)	0/19 (0%)	0.68	3/29 (10.3%)	0/25 (0%)	0.40
2-4 times per month	8/20 (40%)	6/19 (31.6%)		7/29 (24.1%)	4/25 (16%)	
5-8 times per month	3/20 (15.6%)	6/19 (31.6%)		6/29 (20.7%)	7/25 (28%)	
9-16 times per month	4/20 (21.1%)	3/19 (15.8%)		4/29 (13.8%)	6/25 (24%)	
≥ 17 times per month	5/20 (25%)	4/19 (21.1%)		9/29 (31%)	8/25 (32%)	

Table 5-2 continued.

Abbreviations: No., number; SD, standard deviation, N/A, non-applicable, yrs., years.
* = Statistically significant at $p<0.05$. **= Statistically significant at $p<0.10$.

[a] Data is provided for the original study sample although the number of participants varied for the three comparison years as a result of the number of successfully geocoded residences.
[b] Occupation was classified according to 1980 Census Industrial and Occupational Classification Codes .
[c] The analysis excluded two controls who did not hold jobs as of the reference date.
[d] Only participants drinking alcohol once a month or more were included. This involved a total of 20 cases and 19 controls aged 18-54, and 29 cases and 25 controls aged 55 years or older

Table 5-3. The association of concentrations of HAPs characterized by NATA and ALS among age, sex, and race-matched cases and controls in Western Pennsylvania and the Greater Philadelphia area during: 1999, 2002, and 2005.

	Mean (μg/m^3) ± SD Paired t, *p*					
	1999 (n=36 pairs)		2002 (n=45 pairs)		2005 (n=45 pairs)	
Exposure	**Cases**	**Controls**	**Cases**	**Controls**	**Cases**	**Controls**
Metals (N=7)						
Arsenic compounds	0.00024 ± 0.00021 *p*=0.41	0.00021 ± 0.00019	0.0011 ± 0.00034 *p*=0.69	0.0011 ± 0.00039	0.0011 ± 0.00036 *p*=0.35	0.0011 ± 0.0003
Cadmium compounds	0.00013 ± 0.00019 *p*=0.18	0.000095 ± 0.000095	0.00021 ± 0.00021 *p*=0.36	0.00018 ± 0.000079	0.00015 ± 0.000055 *p*=0.57	0.0002 ± 0.00004
Lead compounds	0.0042 ± 0.0033 *p*=0.56	0.0049 ± 0.0064	0.0047 ± 0.0036 *p*=0.87	0.0045 ± 0.0021	0.0040 ± 0.0019 *p*=0.49	0.0038 ± 0.0010
Manganese compounds	0.0018 ± 0.0014 *p*=0.40	0.0015 ± 0.0015	0.0048 ± 0.0056 *p*=0.75	0.0044 ± 0.0061	0.0018 ± 0.0006 *p*=0.54	0.0019 ± 0.00073
Mercury compounds	0.0018 ± 0.00028 *p*=0.92	0.0018 ± 0.00018	0.000054 ± 0.000025 *p*=0.58	0.000051 ± 0.000028	0.000059 ± 0.000038 *p*=0.43	0.000066 ± 0.000042
Nickel compounds	0.0022 ± 0.0023 *p*=0.92	0.0022 ± 0.0018	0.0013 ± 0.00096 *p*=0.44	0.0016 ± 0.0021	0.0010 ± 0.0006 *p*=0.62	0.0011 ± 0.0007
Selenium compounds	0.0045 ± 0.0036 *p*=0.75	0.0047 ± 0.0026	0.00051 ± 0.00024 *p*=0.18	0.00046 ± 0.00016	0.00048 ± 0.00032 *p*=0.84	0.00050 ± 0.00020
Aromatic Solvents[b] (N=6)						
2,4-Dinitrotoluene	0.0000092 ± 0.000022 *p*=0.45	0.0000053 ± 0.000019	3.24 ± 2.50 *p*=0.08**	4.09 ± 2.20	0.000016 ± 0.000026 *p*=0.43	0.000012 ± 0.000018

Table 5-3 continued.

Exposure	Mean (μg/m^3) ± SD Paired t, *p* 1999 (n=36 pairs) **Cases**	 **Controls**	 2002 (n=45 pairs) **Cases**	 **Controls**	 2005 (n=45 pairs) **Cases**	 **Controls**
Benzene	1.53 ± 0.53 *p*=0.41	1.44 ± 0.39	1.69 ± 0.52 *p*=0.045*	1.51 ± 0.36	1.08 ± 0.31 *p*=0.79	1.09 ± 0.24
Ethyl benzene	0.47 ± 0.24 *p*=0.17	0.40 ± 0.17	0.39 ± 0.18 *p*=0.02*	0.31 ± 0.12	0.15 ± 0.09 *p*=0.88	0.15 ± 0.07
Styrene	0.059 ± 0.060 *p*=0.10	0.039 ± 0.026	0.057 ± 0.045 *p*=0.01*	0.037 ± 0.018	0.054 ± 0.055 *p*=0.07**	0.037 ± 0.020
Toluene	3.54 ± 4.44 *p*=0.19	2.48 ± 1.04	3.64 ± 1.36 *p*=0.02*	3.09 ± 0.94	2.15 ± 0.85 *p*=0.99	2.15 ± 0.67
Xylene	1.96 ± 0.93 *p*=0.18	1.69 ± 0.66	1.61 ± 0.81 *p*=0.03*	1.29 ± 0.56	0.738 ± 0.536 *p*=0.99	0.738 ± 0.463
Organic/ Chlorinated Solvents[c] (N=13)						
1,1,2,2-Tetrachloroethane	0.070 ± 0.013 *p*=0.54	0.072 ± 0.011	0.0035 ± 0.00061 *p*=0.35	0.0033 ± 0.00053	0.0033 ± 0.0001 *p*=0.38	0.0034 ± 0.0001
1,1,1-Triochloroethane (Methyl chloroform)	1.36 ± 0.18 p=0.62	1.38 ± 0.16	0.26 ± 0.11 *p*=0.25	0.24 ± 0.049	0.250 ± 0.074 *p*=0.50	0.256 ± 0.070
Tetrachloroethylene (Perchloroethylene)	0.23 ± 0.13 *p*=0.42	0.21 ± 0.057	0.19 ± 0.10 *p*=0.34	0.17 ± 0.07	0.202 ± 0.112 *p*=0.96	0.204 ± 0.089
Carbon disulfide	0.080 ± 0.047 *p*=0.45	0.088 ± 0.050	0.0056 ± 0.0066 *p*=0.26	0.0084 ± 0.016	0.004 ± 0.005 *p*=0.82	0.004 ± 0.004
Carbon tetrachloride	0.27 ± 0.008 *p*=0.50	0.27 ± 0.018	0.61 ± 0.0006 *p*=0.50	0.61 ± 0.00045	0.610 ± 0.001 *p*=0.43	0.610 ± 0.0004
Chloroform	0.087 ± 0.069 *p*=0.83	0.084 ± 0.024	0.098 ± 0.049 *p*=0.89	0.097 ± 0.028	0.085 ± 0.021 *p*=0.75	0.089 ± 0.023

Table 5-3 continued.

Exposure	Mean (μg/m^3) ± SD Paired t, *p* 1999 (n=36 pairs) **Cases**	 **Controls**	 2002 (n=45 pairs) **Cases**	 **Controls**	 2005 (n=45 pairs) **Cases**	 **Controls**
Cresols and cresylic Acid	0.038 ± 0.0060 *p*=0.23	0.036 ± 0.0090	0.0046 ± 0.0021 *p*=0.57	0.0045 ± 0.0022	0.005 ± 0.004 *p*=0.73	0.005 ± 0.002
Ethylene oxide	0.0031 ± 0.0029 *p*=0.87	0.0033 ± 0.0032	0.0052 ± 0.0030 *p*=0.98	0.0052 ± 0.0028	0.005 ± 0.003 *p*=0.30	0.005 ± 0.003
Hexane	0.63 ± 0.33 *p*=0.55	0.59 ± 0.26	0.36 ± 0.17 *p*=0.01*	0.29 ± 0.11	0.158 ± 0.094 *p*=0.83	0.154 ± 0.069
Methyl chloride (Chloromethane)	1.21 ± 0.0084 *p*=0.24	1.21 ± 0.023	1.20 ± 0.0027 *p*=0.096**	1.21 ± 0.0034	1.21 ± 0.003 *p*=0.08**	1.21 ± 0.004
Methylene chloride (Dichloromethane)	0.49 ± 0.26 *p*=0.75	0.48 ± 0.12	0.28 ± 0.082 *p*=0.56	0.27 ± 0.06	0.277 ± 0.075 *p*=0.97	0.282 ± 0.068
Trichloroethylene	0.109 ± 0.058 *p*=0.59	0.104 ± 0.038	0.086 ± 0.048 *p*=0.70	0.090 ± 0.061	0.088 ± 0.045 *p*=0.63	0.091 ± 0.052
Vinyl chloride	0.069 ± 0.017 *p*=0.36	0.072 ± 0.013	0.00052 ± 0.0015 *p*=0.74	0.00064 ± 0.0021	0.00022 ± 0.00039 *p*=0.42	0.0003 ± 0.0008
Other HAPs[d] (N=6)						
Acrylamide	0.00000025 ± 0.00000041 *p*=0.78	0.00000023 ± 0.00000059	0.0000074 ± 0.000016 *p*=0.48	0.0000074 ± 0.000016	0.000000059 ± 0.000000071 *p*=0.10	0.00000009± 0.00000009
Allyl chloride	0.0000019 ± 0.0000086 *p*=0.88	0.0000017 ± 0.0000074	N/A	N/A	0.000006 ± 0.00001 *p*=0.32	0.0016 ± 0.0104
Cyanide	0.095 ± 0.046 *p*=0.16	0.12 ± 0.12	0.069 ± 0.040 *p*=0.15	0.084 ± 0.053	0.074 ± 0.040 *p*=0.16	0.091 ± 0.009
Hexachloroethane	0.0048 ± 0.000014 *p*=0.25	0.0048 ± 0.000013	0.000000012 ± 0.000000028 *p*=0.55	0.0000000089 ± 0.000000023	0.00000045 ± 0.00000054 *p*=0.24	0.0000003 ± 0.0000003

Table 5-3 continued.

Exposure	Mean (μg/m^3) ± SD Paired t, *p* 1999 (n=36 pairs)		2002 (n=45 pairs)		2005 (n=45 pairs)	
	Cases	**Controls**	**Cases**	**Controls**	**Cases**	**Controls**
Hydrazine	0.000000013 ± 0.000000018 *p*=0.64	0.000000017 ± 0.000000051	0.000053 ± 0.000024 *p*=0.75	0.000054 ± 0.000027	0.000054 ± 0.000025 *p*=0.61	0.000065 ± 0.000026
Polychlorinated biphenyls (PCBs)	0.00043 ± 0.000037 *p*=0.51	0.00043 ± 0.000048	0.000040 ± 0.000039 *p*=0.52	0.000036 ± 0.000037	0.000051 ± 0.000066 *p*=0.17	0.000035 ± 0.000039
Pesticides (N=3)						
Ethylene dibromide	0.031 ± 0.007 *p*=0.58	0.031 ± 0.006	0.0011 ± 0.00027 *p*=0.37	0.0011 ± 0.00034	0.0011 ± 0.00026 *p*=0.86	0.0011 ± 0.00029
Ethylene dichloride (1,2-dichloroethane)	0.046 ± 0.011 *p*=0.99	0.047 ± 0.0066	0.0030 ± 0.00061 *p*=0.18	0.0029 ± 0.00085	0.0029 ± 0.0006 *p*=0.65	0.0030 ± 0.00068
Hexachlorobenzene	0.00000047 ± 0.0000003 *p*=0.40	0.00000041 ± 0.0000004	0.00000035 ± 0.00000031 *p*=0.53	0.00000031 ± 0.00000029	0.00000045 ± 0.00000054 *p*=0.24	0.00000033 ± 0.00000005

Abbreviations: NATA, National-Scale Air Toxics Assessment; PM, particulate matter; HAPs, Hazardous Air Pollutants, SD, standard deviation; N/A, not applicable.

* = Statistically significant at $p<0.05$. **= Statistically significant at $p<0.10$.

Table 5-4. ORs (95% CIs) calculated by conditional logistic regression for 3rd and 4th quartile HAPs concentrations by age, sex, and race-matched case-control status for 1999, 2002, and 2005

Table 5-4. ORs (95% CI) calculated by conditional logistic regression for 3rd and 4th quartile [a] HAPs concentrations by age, sex, and race-matched case-control status for 1999, 2002, and 2005.

	1999 (n=36 pairs)	2002 (n=45 pairs)	2005 (n=45 pairs)
Exposure groups[b]	Above median (third and fourth quartiles) OR (95% CI)	Above median (third and fourth quartiles) OR (95% CI)	Above median (third and fourth quartiles) OR (95% CI)
Metals[c]	1.38 (0.55, 3.42)	1.00 (0.38, 2.66)	0.88 (0.32, 2.41)
Aromatic Solvents[d]	1.33 (0.46, 3.84)	1.50 (0.53, 4.21)	0.64 (0.25. 1.64)
Organic/ Chlorinated Solvents[e]	0.36 (0.12, 1.14)	0.42 (0.15, 1.18)	0.50 (0.20, 1.24)
Other HAPs[f]	2.17 (0.82, 5.70)	1.10 (0.47, 2.59)	1.20 (0.52, 2.78)
Pesticides[g]	1.60 (0.52, 4.89)	3.17 (1.27, 7.93)*	0.67 (0.27, 1.63)

Abbreviations: ORs, odds ratios; CI, confidence interval; HAPs, hazardous air pollutants; N/A, not applicable.

* = Statistically significant at $p<0.05$.

[a] The first and second quartiles served as the reference group.
[b] Estimated concentrations with very little variability across residential census tracts were excluded.
[c] Metals include: arsenic, cadmium, lead, manganese, mercury, nickel, and selenium.
[d] Aromatic solvents include: 2,4-dinitrotoluene, benzene, ethyl benzene, styrene, toluene, and xylene.
[e] Organic/chlorinated solvents include: 1,1,1-trichloroethane (methyl chloroform), 1,1,2,2-tetrachloroethane, carbon disulfide, carbon tetrachloride, chloroform, cresols and cresylic acid, ethylene oxide, hexane, methyl chloride, methylene chloride, tetrachloroethylene (perchloroethylene), trichloroethylene, and vinyl chloride.
[f] Other HAPs include: acrylamide, allyl chloride, cyanide compounds, hexachloroethane, hydrazine, and polychlorinated biphenyls (PCBs).
[g] Pesticides include: ethylene dibromide, ethylene dichloride, and, hexachlorobenzene.

Table 5-5. 1999 Final model: ORs (95% CIs) calculated by conditional logistic regression for groups of hazardous air pollutants comparing high (3 & 4) vs. low (1 & 2) quartile exposure by age, sex, and race-matched cases and controls adjusted for smoking (ever/never) and education (<=high school, >high school).

Table 5-5. 1999 Final model: ORs (95% CI) calculated by conditional logistic regression for groups of hazardous air pollutants comparing high (3 & 4) vs. low (1 &2) quartile exposure by age, sex, and race-matched case-control status adjusted for smoking (ever/never) and education (<=high school, >high school).

	B	SE	Exp (B)	95% CI for Exp(B)	
				Lower	B
Metals	0.447	0.899	1.564	0.269	9.107
Aromatic solvents	2.691	1.348	14.745*	1.050	206.976
Organic Solvents	-2.880	1.175	0.056	0.006	0.562
Other Haps	-0.863	0.649	0.056	0.006	0.562
Pesticides	1.257	0.618	3.516*	1.048	11.795
Education	0.892	0.749	2.441	0.562	10.601
Smoking	0.287	0.550	1.332	0.453	3.919

* = Statistically significant at $p<0.05$

Abbreviations: CI, confidence interval; B, beta; SE, standard error; Exp (B), exponentiated beta (odds ratio).

Table 5-6. 2002 Final model: ORs (95% CI) calculated by conditional logistic regression for groups of hazardous air pollutants comparing high (3 & 4) vs. low (1 &2) quartile exposure by age, sex, and race-matched case-control status adjusted for smoking (ever/never) and education (<=high school, >high school).

	B	SE	Exp (B)	95% CI for Exp(B)	
				Lower	Upper
Metals	-0.490	0.919	0.613	0.101	3.715
Aromatic solvents	1.867	1.375	6.469	0.437	95.847
Organic Solvents	-2.059	1.129	0.128	0.014	1.166
Other Haps	0.064	0.663	1.066	0.291	3.905
Pesticides	0.740	0.741	2.095	0.490	8.956
Education	-0.219	0.640	0.803	0.229	2.815
Smoking	0.305	0.539	1.356	0.472	3.899

* = Statistically significant at $p<0.05$

Pesticides	1.257	0.618	3.516	1.048	11.795
Education	0.892	0.749	2.441	0.562	10.601
Smoking	0.287	0.550	1.332	0.453	3.919

* = Statistically significant at $p<0.05$

Table 5-6. 2002 Final model: ORs (95% CIs) calculated by conditional logistic regression for groups of hazardous air pollutants comparing high (3 & 4) vs. low (1 & 2) quartile exposure by age, sex, and race-matched cases and controls adjusted for smoking (ever/never) and education (<=high school, >high school).

Table 5-6. 2002 Final model: ORs (95% CI) calculated by conditional logistic regression for groups of hazardous air pollutants comparing high (3 & 4) vs. low (1 &2) quartile exposure by age, sex, and race-matched case-control status adjusted for smoking (ever/never) and education (<=high school, >high school).

	B	SE	Exp (B)	95% CI for Exp(B)	
				Lower	Upper
Metals	-0.490	0.919	0.613	0.101	3.715
Aromatic solvents	1.867	1.375	6.469	0.437	95.847
Organic Solvents	-2.059	1.129	0.128	0.014	1.166
Other Haps	0.064	0.663	1.066	0.291	3.905
Pesticides	0.740	0.741	2.095	0.490	8.956
Education	-0.219	0.640	0.803	0.229	2.815
Smoking	0.305	0.539	1.356	0.472	3.899

Abbreviations: CI, confidence interval; B, beta; SE, standard error; Exp (B), exponentiated beta (odds ratio).

Table 5-7. 2005 Final model: ORs (95% CIs) calculated by conditional logistic regression for groups of hazardous air pollutants comparing high (3 & 4) vs. low (1 & 2) quartile exposure by age, sex, and race-matched cases and controls adjusted for smoking (ever/never) and education (<=high school, >high school).

Table 5-7. 2005 Final model: ORs (95% CIs) calculated by conditional logistic regression for groups of hazardous air pollutants comparing high (3 & 4) vs. low (1 & 2) quartile exposure by age, sex, and race-matched case-control status adjusted for smoking (ever/never) and education (<=high school, >high school).

	B	SE	Exp (B)	95% CI for Exp(B)	
				Lower	B
Metals	0.493	0.681	1.637	0.431	6.215
Aromatic solvents	-0.129	0.749	0.879	0.203	3.815
Organic Solvents	-0.691	0.681	0.501	0.132	1.904
Other Haps	0.398	0.561	1.489	0.496	4.469
Pesticides	-0.621	0.568	0.538	0.176	1.638
Education	-0.193	0.49	0.825	0.316	2.153
Smoking	-0.247	0.47	0.781	0.311	1.963

Abbreviations: CI, confidence interval; B, beta; SE, standard error; Exp (B), exponentiated beta (odds ratio).

6.0 CONCLUSIONS

This dissertation was designed to explore the association of personal risk factors and environmental and occupational exposures and ALS based on results from our case-control study as well as from existing data. It has been separated into three complementary topics as follows: 1) risk of ALS with exposure to pesticides; 2) risk of ALS with exposure to personal, environmental, and occupational risk factors; and 3) risk of ALS with exposure to hazardous air pollutants.

Previous studies have reported associations between a number of personal risk factors and environmental and occupational exposures and risk of ALS; however, the results have been inconsistent. Our meta-analysis found a relationship between exposure to pesticides and risk of ALS among all cases and male cases compared to controls. We failed to find an association between pesticide exposure and risk of ALS among females, possibly due to the small number of studies (n=3) and few women in our analysis. The potential relationship between pesticide exposure and ALS has been difficult to establish as most studies have failed to obtain details regarding class of pesticide class (insecticide, herbicide, fungicide, etc.), chemical, and duration of exposure. Grouping all classes of pesticides together can also be problematic as the effect of one class may be diluted resulting in a lack of an association. In our analysis, the majority of studies reported exposure to agricultural occupational chemicals but did not specify the

chemicals or jobs involved; however, our findings indicate that men may be more likely to be occupational exposed to pesticides and for longer periods of time than women.

Future studies with more accurate exposure assessments or job exposure matrices are needed to further explore this relationship. Protective equipment should always be worn and precautionary measures taken to prevent the potential for "take-home" exposures to others. In summary, more research is needed to determine whether an association truly does exist between suspected pesticide exposure and risk of ALS.

A gene-environment etiology is suspected for ALS; however, results from previous studies have been inconsistent. Our case-control study found an association between occupational exposure to metals and pesticides and elevated risk of ALS after adjusting for smoking and education. These findings confirm those of others suggesting an association between metal exposure and ALS (Felmus, Patten et al. 1976; Conradi, Ronnevi et al. 1978; Armon, Kurland et al. 1991; Chancellor, Slattery et al. 1993) and between pesticide exposure and ALS (McGuire, Longstreth et al. 1997; Kamel and Hoppin 2004; Govoni, Granieri et al. 2005; Morahan and Pamphlett 2006; Bonvicini, Marcello et al. 2010) Our results suggest a possible link between occupations involving exposure to metals or pesticides and risk of ALS.

As the majority of exposure assessment is obtained through self-report without verification of records or biological samples obtained, it can be difficult to accurately assess the quantity, frequency, and duration of occupational exposures. Possible reasons for inconsistent results among previous studies include: varying definitions of exposures or occupations as well as different classifications of occupations by the various coding methods or years. Limitations of case-control study designs include recall bias and selection bias; however, matching in control selection greatly reduces the possibility for confounding of the factors matched upon. The

results of our study are not generalizable to the general population as outpatient hospital controls were used, but nevertheless, the results are an important finding. Additional research is needed with a larger sample size, population-based controls, and the inclusion of other races/ethnic groups to further examine the association of occupational exposure to metals and pesticides and risk of ALS.

The EPA's NATA data was examined in our third study to explore the potential relationship of elevated ambient air concentrations of hazardous pollutants and risk of ALS. Concentrations of individual compounds as well concentrations of groups of structurally similar compounds (i.e., metals, aromatic solvents, pesticides, etc.) were assessed for three years of NATA data: 1999, 2002, and 2005. A relationship was found between exposure to ambient air concentrations of hazardous pollutants by census tract of residence and risk of ALS, while adjusting for education and smoking. More specifically, exposure to solvents and pesticides was associated with increased risk of ALS among cases and matched controls by the 1999 NATA assessment.

Although concentrations of compounds were not available on an individual level, these ambient air estimates are believed to be valid measures of exposure (Payne-Sturges, Burke et al. 2004). Our findings varied by year of NATA assessment due possibly to real changes in emissions or source characterization, advancements in methodology, equipment used, or changes in climate, weather, industries, automobile fuel combustion, or power generation; however, these findings are noteworthy as an association was found for exposure to hazardous air pollutants and a new area of ALS research.

In conclusion, a number of environmental and occupational exposures were related to risk of ALS. Our meta-analysis found an association of pesticide exposure and ALS. Using data available through the EPA, an association was found for exposure to ambient air concentrations

of solvents and pesticides by census tract of residence and risk of ALS. In addition, occupational exposure to pesticides and metals were found to be associated with ALS through our case-control study. ALS is a complex disease that is both debilitating and fatal. Further research is needed to investigate the relationship between personal risk factors and environmental and occupational exposures and risk of ALS.

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