DISSERTATION

DIAGNOSED DEPRESSION AND LOW, INTERMEDIATE, AND HIGH PESTICIDE EXPOSURES IN IOWA AND NORTH CAROLINA FARM APPLICATORS AND THEIR SPOUSES ENROLLED IN THE AGRICULTURAL HEALTH STUDY

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY CHERYL LYNN BESELER ENTITLED "DIAGNOSED DEPRESSION AND LOW, INTERMEDIATE, AND HIGH PESTICIDE EXPOSURES IN IOWA AND NORTH CAROLINA FARMER PESTICIDE APPLICATORS AND THEIR SPOUSES ENROLLED IN THE AGRICULTURAL HEALTH STUDY" BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

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ABSTRACT OF DISSERTATION

DIAGNOSED DEPRESSION AND LOW, INTERMEDIATE, AND HIGH PESTICIDE EXPOSURES IN IOWA AND NORTH CAROLINA FARMER APPLICATORS AND THEIR SPOUSES ENROLLED IN THE AGRICULTURAL HEALTH STUDY

The purpose of this study was to evaluate the relationship between history of acute pesticide poisoning and diagnosed depression. The study population came from the Agricultural Health Study, which enrolled 52,395 farmer applicators and 32,347 of their spouses from 1993 to 1997 in Iowa and North Carolina. Data were obtained by self-administered questionnaires and included information on farm characteristics, pesticide exposures, medical conditions, and demographic and lifestyle factors. Cases were defined as those who reported having ever been diagnosed with depression. Exposures were based on total lifetime days of mixing or applying pesticides and reported poisonings. The study included 20,553 male farmer applicators and 29,074 female spouses.

A total of 3.1% of farmers and 7.1% of the female spouses reported a diagnosed depression. Separate analyses on farm applicators and their spouses showed pesticide poisoning to be significantly associated with depression (OR for female spouses 3.28; 95% CI 1.73, 6.22 and OR for farm applicators 3.83; CI 2.63, 5.60). The findings from the two analyses support past epidemiological studies reporting that a high-level exposure or acute pesticide poisoning is associated with depression. The associations remained significant in separate analyses by state. Among farm spouses, where only 50% of the study population applied pesticides, involvement of certain OPs and carbamate compounds was apparent.

Future epidemiological studies should obtain complete histories of affective disorders prior to the pesticide poisoning, as well as an inventory of symptoms appearing after poisonings. Future studies should compare those who have developed depressive symptoms after a pesticide poisoning to those who did not develop depressive symptoms after a poisoning. Mood disorders may be a marker of other neurological effects, all of which may have the same underlying biochemical mechanism.

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TABLE OF CONTENTS

INTRODUCTION

This dissertation is formatted as four chapters containing a background chapter, two separate papers, and a conclusion. Chapter one will present the biological foundation for the papers and provide biochemical and toxicological evidence of a link between organophosphorus compounds and depression. Chapter two, the first paper, will provide epidemiological evidence for the association of exposure to organophosphate pesticides and depression among female spouses of farm pesticide applicators. Chapter three, the second paper, will provide further evidence of this connection by examining depression and acute pesticide poisoning in male farmer applicators. Chapter four is the conclusion of the two studies, addressing how they support previous epidemiological studies and the underlying biological hypotheses. Ideas for future research directions in the field of pesticide epidemiology are discussed in light of these findings. Appendices include questionnaires from which the data were derived for this study.

The biological basis for this study of acute pesticide poisoning and depression begins with the history of organophosphorus compounds developed as nerve gas agents and later found to be beneficial in the protection of crops from insect pests. They are a diverse group of compounds with varying degrees of toxicity. Acute toxicity is due to inhibition of acetylcholine esterase, but targets resulting in chronic effects have yet to be fully characterized. Certainly, genetic susceptibility to pesticide poisoning, such as having a polymorphic paraoxonase gene, or having a history of affective disorders, contributes to the neuropsychiatric outcome of a poisoning. The characterization of a putative target of organophosphates, neuropathy target esterase, as a lysophospholipase has strengthened the possibility of a connection between hypercholinergic responses and

depression because its inhibition increases the available choline in the cell and it is involved in signal transduction. Patients with past or active affective disorders are more sensitive to agents that stimulate cholinergic activity possibly exacerbating the effects of excess acetylcholine, or its precursor, choline.

Chapter two describes a study of female spouses of farm applicators, who have been overlooked in past epidemiological studies because farming is not considered their primary occupation and they are not viewed as pesticide applicators. The primary purpose of this study was to determine whether low, intermediate, or high levels of pesticide exposure was associated with diagnosed depression in a large cohort of women farm residents. A second objective was to determine whether organophosphates or carbamates were the likely agents associated with any positive association observed. The study population came from the 32,347 spouses of private applicators enrolled into the Agricultural Health Study (AHS) between 1993 and 1997 in Iowa and North Carolina. After exclusions, the study population consisted of 29,074; 2,051 cases of diagnosed depression and 27,023 controls. There were 20,073 women in Iowa and 9,001 women in North Carolina.

Based on a case-control study design, cases were defined as those who responded yes to a doctor having diagnosed depression for which medication was required. Pesticide exposure was calculated as lifetime days of use from number of years multiplied by days per year they personally mixed or applied pesticides. Those who reported never mixing or applying pesticides comprised the reference group. The low-level exposure group mixed or applied fewer than 226 days, below the 90th percentile. The intermediate-level exposure group mixed or applied at least 226 days. The high

exposure group was reserved for those who reported a diagnosed pesticide poisoning. Covariates included state of residence, age, race, Hispanic ethnicity, education, having a job off the farm, alcohol use and cigarette smoking. The number of doctor visits was used to adjust for potential diagnosis bias. A propensity score, representing the probability of having any of 38 medical conditions and being diagnosed with depression, was used to adjust for the association of poor health and depression. Univariate and multivariable logistic regression analysis was performed on pesticide exposure levels and diagnosed depression. Principal component analysis (PCA) was used to identify factors correlated with specific pesticides and significant factors were tested in logistic regression models to determine whether they were associated with depression.

Cases differed from controls in that they were more likely to live in North Carolina, less likely to be white, and more likely to work a job off the farm. They were more likely to be between 40 and 59 years of age, have visited the doctor at least once in the previous year, were past or current cigarette smokers, and likely to abstain from drinking alcohol. The propensity score indicated that they were more likely to have a medical condition than controls. After adjusting for these potential confounders, having a diagnosed pesticide poisoning showed a much stronger association with diagnosed depression than low or intermediate exposure levels (OR 3.28; 95% CI 1.73, 6.22). The effect was stronger in North Carolina (OR 5.25; CI 1.81, 15.2) than in Iowa (OR 2.43; CI 1.05, 5.62). Using factors obtained from the PCA analysis, OPs and carbamates were significantly associated with depression, and the effect was stronger in women in North Carolina than in Iowa.

A similar analysis of farmer applicators in the AHS is presented in Chapter three. The primary purpose of this study was to characterize diagnosed depression in five categories of pesticide exposure ranging from low-level exposure to a pesticide poisoning in a large group of farm pesticide applicators. The study population came from the 22,916 private farm applicators enrolled into the AHS in 1993 through 1997 in Iowa and North Carolina who returned the take-home questionnaire. After exclusions, the study population consisted of 20,553; 644 cases of diagnosed depression and 19,909 controls. There were 13,632 applicators in Iowa and 6,921 in North Carolina.

Using a case-control study design, cases were defined as those who responded yes to a doctor having diagnosed depression requiring medication or shock therapy. Pesticide exposure was calculated as total lifetime days of use from number of years multiplied by days per year they personally mixed or applied pesticides. Those who reported mixing or applying pesticides fewer than 225 days, the median, comprised the reference group. The intermediate-level exposure group mixed or applied between 224 and 753 days, below the 90th percentile. The high-level exposure group mixed or applied at least 753 days, or above the 90th percentile. The unusually high exposure group and those who reported a diagnosed pesticide poisoning comprised the two highest exposure groups. Covariates included state of residence, age, race, Hispanic ethnicity, education, having a job off the farm, not working on the farm or not having crops or animals, alcohol use and cigarette smoking. The number of doctor visits was used to adjust for potential diagnosis bias. A propensity score, representing the probability of having any of 37 medical conditions and being diagnosed with depression, was used to adjust for the association of poor health

and depression. Univariate and multivariable logistic regression analysis was performed on pesticide exposure levels and diagnosed depression.

Cases differed from controls in that they were more likely to live in Iowa, and less likely to be involved in farm work. They were older, a past or current smoker, more likely to abstain from drinking alcohol, and to have had at least one visit to the doctor in the previous year. After adjusting for these potential confounders, having a diagnosed pesticide poisoning showed a much stronger association with diagnosed depression than low, intermediate, high or unusually high exposure levels (OR 3.83; 95% CI 2.63, 5.60). The estimated effect was stronger in North Carolina (OR 4.29; CI 2.19, 8.40) than in Iowa (OR 3.64; CI 2.29, 5.77).

CHAPTER ONE: BIOLOGICAL PLAUSIBILITY

Overview of the history of organophosphorus compounds

In 1820 Lassaigne reacted ethanol with phosphoric acid and produced triethyl phosphate, the first phosphoric acid derivative (Chambers 1992). The first organophosphorus (OP) compound was synthesized in 1854 when de Clermont made tetraethyl pyrophosphate (TEPP), but it was 80 years later when the insecticidal properties of TEPP were discovered by Gerhard Schrader (Chambers 1992). Triesters of phosphoric acid were manufactured in the 1940's as by-products of nerve gases, such as soman, sarin and tabun, developed by the Germans prior to and during World War II (Chambers et al. 1994; Edmunson 1988). They are pentavalent OP compounds and are structurally different from the trivalent OPs. Although used broadly to represent an entire class of OP compounds, organophosphates are defined specifically as esters and organic acid halides of phosphoric acid (Thompson et al. 2004). Trivalent OPs, triphosphites, are used as antioxidants in industry and as a cotton defoliant (Abou-Donia 1992). Chemical warfare agents have been mostly phosphonates with only one substituent on the phosphorus attached by a phosphate-carbon bond (Marrs 1993). Soman and sarin are fluorine-substituted phosphonates (Marrs 1993). Although not as toxic as nerve gases, approximately 200 OP pesticides act by inhibiting esterases, including acetylcholinesterase, which degrades the nervous system neurotransmitter, acetylcholine (Kwong 2002). Sulfur-containing analogs of OP compounds have their sulfur atom replaced with oxygen during metabolism, producing a more toxic compound (Chambers 1992).

OP compounds, such as diisopropyl fluorophosphate (DFP), have been useful to researchers in the study of cholinesterase function in both health and disease (Grob 1950). OP compounds have been used to treat medical conditions, such as abdominal distention, urinary retention and glaucoma (Grob et al. 1947; Leopold et al. 1946). Although used throughout the world as an insecticide and also associated with fatal poisonings, TEPP has shown to be useful in managing myasthenia gravis (Grob et al. 1949).

Hydrolysis is common to all ester compounds such that any number of esterases in mammalian systems will hydrolyze OP compounds at the leaving group, but with great variability in the rate of reaction. Acetylcholine esterase (AChE), butyrylcholinesterase (BuChE), neuropathy target esterase (NTE), and other esterases may be targets of OPs. AChE inhibition, but not BuChE inhibition, explains the acute toxicity of OPs (Chen et al. 1999), while NTE is the putative agent causing OP-induced delayed neuropathy (OPIDN) (Richardson 1992). Many potential, but as yet uncharacterized, targets exist to explain the reported intermediate and long-term effects of OP intoxication (Ray et al. 2001). Chanda (1997) showed that liver carboxylesterases are more sensitive to chlorpyrifos than are AChEs in rats (Chanda et al. 1997). Richards et al. (2000) identified acylpeptide hydrolase, a neuropeptide metabolic enzyme, which shows great activity towards dichlorvos and DFP and produces cognitive effects (Richards et al. 2000). Fatty acid amide hydrolase is a serine hydrolase whose substrates include neuromodulatory fatty acids and has structural similarity to NTE (Atkins et al. 2000). This integral membrane protein has recently been shown to be involved in the degradation of members of the endocannabinoid class of signaling lipids that modulate

pain, cognition, feeding, sleep and locomotor activity (Bracey et al. 2002). There are more than 100 lipid hydrolases (lipases) expressed in the human proteome which may have activity towards any number of OP compounds (Bracey et al. 2002). Lipases are known to be involved in a number of diseases as a result of their role in lipid metabolism and signal transduction (Wang et al. 1999).

OP compounds are used in large quantities all over the world. Although accurate numbers are lacking, the World Health Organization (WHO) estimated that there were more than three million OP poisonings in the world and 200,000 deaths resulting from these OP poisonings in 1985 (Jeyaratnam 1990). Two-thirds of these were hospitalized suicide attempts (Jeyaratnam 1990). This is an increase over the 500,000 acute pesticide poisoning the WHO estimated in 1973 to occur annually worldwide (Jeyaratnam 1990). OP poisonings occur most often in agricultural and manufacturing workers and in small children (O'Malley 1997). In 1999, there were 13,348 OP poisonings and five reported OP-related fatalities in the United States (Litovitz et al. 2000).

Toxicology of organophosphorus compounds

AChE insecticides consist of esters of phosphoric (P=O) or phosphorothioic acids (P=S) and esters of carbamic acid (the carbamates) (Chambers 1992). All OP insecticides have a pentavalent phosphorus atom with a double bond to oxygen or sulfur (Chambers 1992). Those with a sulfur bond are less toxic to mammalian systems than those with a phosphorus bond until they are transformed by cytochrome P450 monooxygenases to the oxon by a desulfuration step (Chambers 1992). It is only then that they can inhibit AChE (Chambers 1992). Substituent groups on the phosphate can be alkyl groups, alkoxy groups, aryl or amido groups (Figure 1) (Crosby 1998). Many

OPs have a group that functions as a leaving group and can be a halogen, such as fluoride, a heterocyclic group or an S-alkyl group (Chambers 1992). The bond from the phosphorus to the leaving group is the most labile and determines the reactivity of the OP with AChE; however, this leaving group may change after the desulfuration step in those compounds with a phosphorus-sulfur double bond (Levi et al. 1992). The structure of the substituent group gives the OP its unique physicochemical properties including its affinity for AChE, the time for hydrolysis of the OP by AChE, and the regenerative capacity of the enzyme, all of which determines toxicity and time to development of symptoms (Chambers 1992; Timbrell 2000).

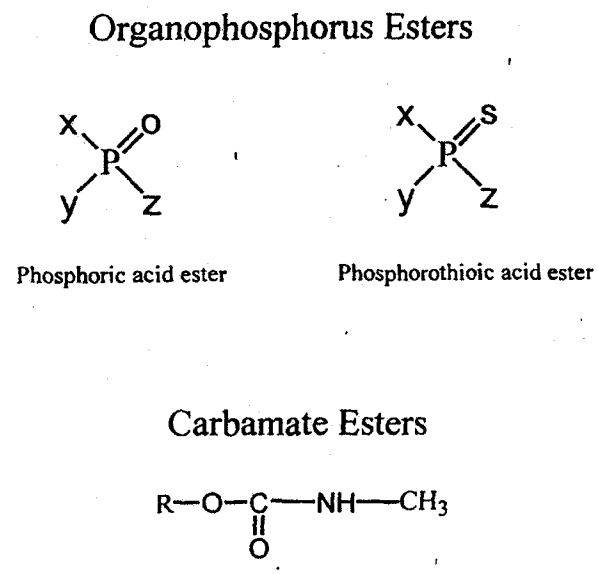


Figure 1. Examples of phosphate, phosphothioate and carbamate esters
X, Y = alkyl, alkoxy, aryl, amido; Z=aryl, alkyl, S-alkyl, alkoxy, halogen;
R=aryl/ alkyl

The goal of detoxification systems is to first make something soluble and reactive so that it can perform the second step which is to create an adequate substrate for linking to biological molecules that can be readily excreted from the body (Timbrell 2000). The

first step is referred to as the Phase I reactions involving the monooxygenase family of P450 enzymes (Timbrell 2000). Phase II involves many different enzymes found in tissues and links the reactive compound to glutathione, for example, so that it can be readily excreted (Timbrell 2000).

The sulfur-containing OPs can contain one or two sulfurs bonded to the phosphorus (Chambers 1992). The phosphorothioate parathion contains one sulfur, and the phosphorodithioate malathion has two sulfurs (Figure 2) (Crosby 1998; Timbrell 2000). OPs such as parathion and malathion become more potent inhibitors of AChE after they are oxidized by cytochrome P450 monooxygenases (Crosby 1998; Timbrell 2000). Oxidative desulfuration of phosphorothioate esters creates a biotransformation product that confers greater chemical stability in the enzyme active site than the parent compound (Levi et al. 1992). This step takes a "thion" and makes an "oxon" (Figure 2) (Levi et al. 1992). The replacement of sulfur with the more electronegative oxygen gives it increased reactivity towards the nucleophilic serine in the active site of the esterase (Crosby 1998; Levi et al. 1992; Timbrell 2000). Toxicity is species specific, insects being more sensitive than mammals and is a function of differential metabolism due to the oxidative transformation and rate of subsequent hydrolytic conversion to a less toxic substance (Chambers 1992).

The monooxygenase system catalyzes many reactions involving substituents on side groups including aromatic ring hydroxylation, thioether oxidation, deamination, alkyl and N-hydroxylation, N-oxide formation, and N-dealkylation (Vale 1998; Levi et al. 1992). These are the Phase I reactions where oxidative, reductive and hydrolytic reactions form reactive metabolites (Levi et al. 1992). The result is a more polar

molecule that can continue on to Phase II reactions (Levi et al. 1992). The metabolite produced from OPs can be hydrolyzed by aryl and aliphatic hydrolases found in mammalian tissues or conjugated with glutathione, glucuronic acid and glycine with the purpose of combining the metabolite with a natural molecule in the organism to enhance water solubility and excretability (Levi et al. 1992).

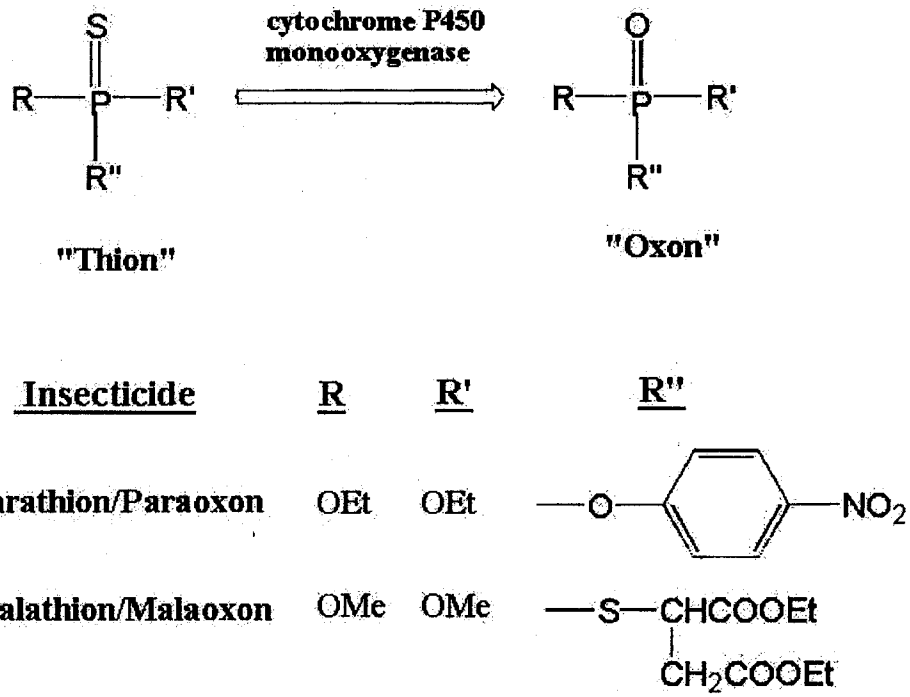


Figure 2. The initial cytochrome P450 monooxygenase reaction converting a sulfur-containing OP to an oxygen-containing OP; examples of OPs which undergo this conversion to make a more toxic metabolite than the parent compound.

Nerve gases and OP pesticides act as pseudosubstrates and form covalent adducts with the active site serine in AChE (Timbrell 2000). Nerve gases are more toxic than OPs because they irreversibly phosphorylate the serine in the active site of AChE, making it extremely resistant to hydrolysis (Marrs 1993). OPs, such as parathion and malathion, contain a phosphorus-sulfur double bond which does not create a permanent

inhibition of the enzyme but an enzyme that can be reactivated at a very slow rate (Marrs 1993). Usually the rate is so slow that a new enzyme is synthesized before the release of phosphate occurs, the half-life of the enzyme being about 10-30 days, making repeated, subtoxic exposures that result in a cumulative response (Timbrell 2000).

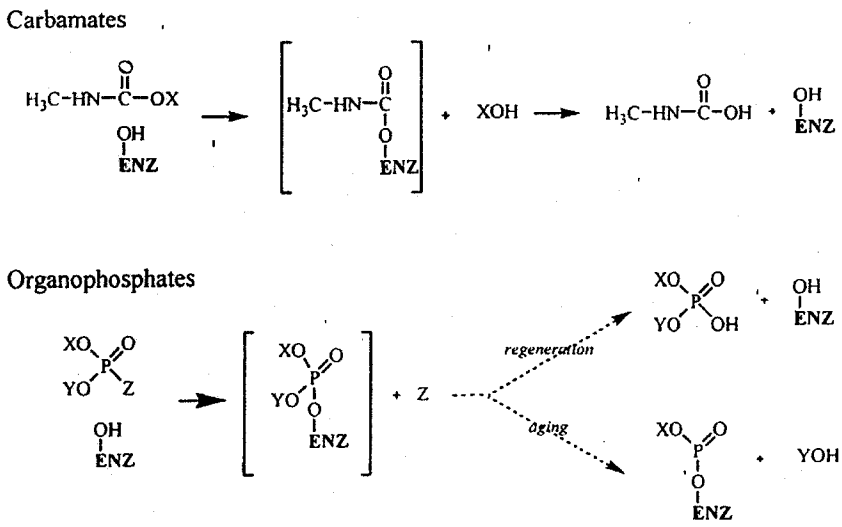


Figure 3. The mechanism by which carbamates and OPs phosphorylate serine 203 in the active site of AChE and form an enzyme intermediate. The complex is more stable than acetylcholine and breakdown of this complex is slow. Aging of the enzyme means the reaction is irreversible.

OPs containing halogenated aryl groups, such as dichlorfenthion and leptophos, have partition coefficients that are similar to organochlorines and may bioaccumulate in adipose tissue (Freed et al. 1976). Partitioning in fat depots is a function of having a high partition coefficient into organic solvents, rather than lipid solubility alone, and a slow rate of hydrolysis during detoxification (Freed et al. 1976). This partitioning into fat may alter the response to toxicity by removing the compound from circulation, allowing it to be released slowly into the circulation and resulting in chronic toxicity.

Most OPs are lipophilic and are not ionized, making absorption rapid following inhalation or ingestion (Vale 1998). Dermal absorption tends to be slower but can be increased with increased contact with skin, with increased lipophilicity of the specific OP, and with solvents present in the OP formulation (Kanikkannan et al. 2000). The physicochemical properties of OPs are directly related to their chemical structures. Those that are more volatile, such as dichlorvos, chlorpyrifos and diazinon, show increased potential for inhalation and a higher rate of dermal absorption. The solvent used and the formulation as a powder or liquid, as well as impurities in the formulation, can all affect toxicity. Co-formulants in the OP mixtures can include organic solvents, detergents, and emulsifying agents. Some OP formulations are more toxic than the OP alone. The solvent used in chlorpyrifos showed a nearly two-fold increase in dermal absorption over chlorpyrifos alone (Griffin et al. 2000). Fatty acids, fatty alcohols and terpenes in the formulation increase dermal absorption of OP pesticides (Kanikkannan et al. 2000).

Work practices and the wearing of personal protective equipment greatly influence the amount of exposure to OP pesticides (Karalliedde et al. 2003). Removing contaminated clothing and discarding or washing it immediately after exposure can reduce the hazards associated with OP use (Karalliedde et al. 2003). Physical exertion to the point of increasing the respiratory rate can increase the amount of inhaled OPs reaching the lungs (Karalliedde et al. 2003). The size of particles and droplets are associated with deposition in the lungs (Moretto et al. 2001). Hand-to-mouth exposures can occur during mixing and spraying, and failure to wash immediately after these activities can result in increased dermal exposure and risk of ingestion. Dermal

penetration varies depending on the part of the body exposed. Exposures to the head area including the face, forehead, scalp and neck are absorbed two to six times more than exposures to the forearm (Maiback et al. 1971). Hydration, temperature, disease and inflammation alter blood flow to the skin and influence dermal absorption (Riviere et al. 1992).

Factors related to the individual, such as age, sex, health status, medications being taken, and cholinergic status, as well as variations in metabolism, sequestration and excretion all affect the toxicity of an OP (Nakajima et al. 2000). The exposure dose is usually based on the concentration of the OP when mixed according to manufacturer's instructions and does not take into account degradation and transformation. For example, diazinon degrades into a product that is 1000-fold more toxic than its parent compound (Soliman et al. 1982).

Hayes et al. (1980) characterized exposure to the OPs dichlorvos, diazinon and chlorpyrifos in 25 pest control operators around Houston, Texas. Measurements were taken using personal and environmental air sampling to obtain an eight-hour average exposure level. Biological monitoring utilized urinalysis to quantify alkyl phosphate metabolites, blood samples were obtained to measure AChE and BuChE activity, and a physical examination was conducted to detect toxic effects of pesticide exposure. The monitoring was performed once a month for three consecutive months in the spring of 1979. The investigators developed the methods to measure airborne levels of OP pesticides and percent recovery from air monitoring devices. They obtained all urine that was voided pre-shift, during the shift and post-shift once for each of the three months of the study. The blood sample results from the study group were compared to 22 age and

gender-matched controls. The physical examination included a complete laboratory blood analysis. The results showed that 96% of those exposed to OPs had measurable metabolites and the study group had a statistically significant inhibition of BuChE compared to the control group ($P<0.001$). The red blood cell AChE levels did not differ between the study and the control groups. The investigators noted that the reduced levels of BuChE might reflect depletion of the body's reserve potential for OP detoxification (Hayes et al. 1980).

Although not understood in 1980, it is presently thought that BuChE, found mainly in neurons and glial cells, may provide a functional pool of AChE and can act to replace AChE in the brain when necessary (Giacobini 2003). The findings reported by Hayes et al. may reflect chronic, low-dose OP exposure and not be reflective of acute effects. Headaches and respiratory problems were the most commonly reported health effects in the cohort studied by Hayes et al. (1980). No one in the study group reported a history of acute OP poisoning. Hayes et al. (1980) concluded that no single measure of exposure was adequate in defining exposure, but all three together provided a reasonable measure for overall exposure.

Physiological effects of exposure to organophosphorus pesticides

Exposure to OP pesticides has been associated most prominently with short-term acute effects resulting from cholinesterase inhibition, but intermediate syndrome and OP-induced delayed neuropathy (OPIDN) have also been observed after acute poisoning incidents (Brown et al. 1998; Jamal 1997; Marrs 1993). Exposure to OPs has historically been measured using AChE inhibition; with a 40% to 50% inhibition of the enzyme, symptoms of acute OP toxicity occur (Kwong 2002). Short-term acute toxic effects are

caused by an inhibition of AChE, an enzyme that inactivates the neurotransmitter, acetylcholine, causing acetylcholine to accumulate at cholinergic synapses (Vale 1998). The result is cholinergic syndrome defined as an overstimulation of muscarinic and nicotinic acetylcholine receptors in the central and peripheral nervous systems. Similar to OP pesticides, carbamate pesticide exposure also results in accumulation of acetylcholine, but carbamates bind reversibly to cholinesterases and readily dissociate from the enzyme (Kwong 2002). Symptoms after poisoning are much less severe because carbamates are more rapidly hydrolyzed than OPs, resulting in faster regeneration of active enzyme (Kwong 2002).

Impairments resulting from pesticide poisoning may include both cognitive and physical sequelae (Jamal 1997). Overstimulation of muscarinic receptors in the parasympathetic autonomic system and postganglionic nerve fibers affects the exocrine glands, eyes, gastrointestinal tract, respiratory tract, cardiovascular system and bladder (Ecobichon 1991). The manifestations of effects on the exocrine glands and eyes include increased salivation, lacrimation, sweating and blurred vision (Ecobichon 1991). Effects on the gastrointestinal tract include nausea, vomiting, abdominal cramps and diarrhea (Ecobichon 1991). Respiratory effects include excessive bronchial secretions, wheezing, edema, tightness in the chest, bronchospasms, and cough (Ecobichon 1991). Acute effects reported include: nausea, vomiting, abdominal pain, numbness and/or tremors in the extremities, fatigue, headaches, excessive salivation, diarrhea, generalized weakness, respiratory problems, and blurred vision (Ellenhorn et al. 1996). Acetylcholine accumulates in the synapse and overstimulation of acetylcholine receptors occurs (Davies 1990). Death usually results from respiratory failure (Davies 1990). Atropine, a

competitive acetylcholine antagonist, can be given after a poisoning to reverse the biochemical effects of excess acetylcholine in the synapse (Kwong 2002; Marrs 1993). Pralidoxime, widely available in the United States, or obidoxime, available in Europe and other parts of the world, both nucleophilic oximes, can be used to regenerate AChE (Kwong 2002; Marrs 1993).

Those who suffer significant cholinesterase inhibition present with weakness of the lower extremities which progresses to the upper extremities; ataxia and paralysis occurs in one to three weeks after intoxication (Abou-Donia 1981). Mild cases usually resolve in several months but in more severe cases symptoms can persist for much longer periods of time (Abou-Donia 1990).

Neuropsychological effects were described in a series of papers published in 1950 (Grob 1950; Wood 1950). As early as 1953, anxiety, withdrawal, and depression were noted after nerve gas poisoning (Grob et al. 1953) but were not fully characterized until 1961 when Gershon and Shaw established specific psychiatric sequelae associated with OP poisoning (Gershon et al. 1961). In 1964 Dille and Smith published two case reports of aerial pesticide applicators who manifested extreme psychiatric disorders thought by the researchers to be associated with OP exposure, one of the pilots having been doused with an OP mixture when his tank overfilled (Dille et al. 1964).

A study of chlorpyrifos in rats noted behavioral alterations from OP administration at doses that did not produce any of the classic signs of cholinergic toxicity (Sanchez-Amate et al. 2001). Epidemiological studies have also measured high rates of extrapyramidal and psychiatric symptoms in those exhibiting no depression of AChE activity (Salvi et al. 2003). Studies done in rats and monkeys suggested that long-

term exposure to low levels of OPs may lead to tolerance and a reduction in clinical effects associated with acute exposures (Prendergast et al. 1998). A compensatory mechanism might be down-regulation or functional adaptation of central muscarinic and nicotinic receptors in response to elevated acetylcholine levels over time (Costa et al. 1982; Prendergast et al. 1998). The most consistent evidence comes from studies by Overstreet et al. (1974) in DFP-tolerant rats showing that tolerance is related to subsensitivity to muscarinic agonists, indicating a down-regulation of muscarinic receptors from either decreased receptor density and/or receptor affinity (Overstreet et al. 1974). It was further shown that choline uptake was decreased in rats and guinea pigs subacutely treated with DFP (Hoskins et al. 1992). Acetylcholine regulates gamma-amino butyric acid (GABA) synthesis such that elevated acetylcholine increases GABAergic-dopaminergic activity and this, in turn, decreases dopamine turnover (Hoskins et al. 1992). These systems appear to respond in an effort to balance the increased levels of acetylcholine in striatal neurons, and play a role in tolerance to chemicals such as DFP (Hoskins et al. 1992).

An alternative outcome to tolerance at subacute doses of OP pesticide is the phenomenon of sensitivity to these compounds after acute or intermittent exposures (Miller 1994). Miller and Mitzel (1995) reported on 112 individuals with onset of multiple chemical sensitivity after exposure to either OP or carbamate pesticides or the remodeling of a building. Those with a documented exposure to OP or carbamate pesticides reported significantly greater symptoms than those exposed to the remodeling of a building (Miller and Mitzel. 1995). Pall (2003) proposed a biochemical mechanism to explain the observation that OPs and carbamates produce a biochemical sensitivity

response; specifically, an increase in NMDA receptor activity results in increased nitric oxide and that this cascade of events results in multiple chemical sensitivity (Pall 2003). Animal models suggest an association between supersensitivity to cholinergic agonists in rats and symptoms of multiple chemical sensitivity in humans (Bell et al. 1992; Overstreet et al. 1996).

Given the short-term effects represented by AChE inhibition and the possible development of tolerance or sensitivity to OPs, using AChE as a sole measure of exposure may not be sufficient to characterize exposure to OPs (Ray et al. 2001). The study by Hayes et al. showed that one measure of OP exposure does not adequately reflect exposure (Hayes et al. 1980). Due to differences in the pharmacokinetics of specific OPs and individual variation in metabolism, metabolite concentrations may not adequately reflect dose (Arbuckle et al. 2002; Ray et al. 2001). Measuring particular metabolites in urine may not be appropriate when the study group applies a number of different OPs. Comparing the results of OP exposures and neurological outcomes from different countries, where OP formulations, work practices and individual characteristics differ, makes it challenging to arrive at any definitive conclusions about the nature of the neurological effects resulting from OP use (Forget 1991; Jeyaratnam 1985).

Physiology of the neurotransmitter acetylcholine

Acetylcholine is the primary neurotransmitter in the central nervous system, both in the somatic motor nervous system and in the autonomic motor nervous system (Patrick 2001). Acetylcholine is synthesized in the presynaptic nerve from choline and acetyl coenzyme A with catalytic assistance from the enzyme choline acetyltransferase (Patrick 2001). Acetylcholine and its specific carrier protein are immediately packaged into

membrane-bound vesicles (Patrick 2001). Upon arrival of a nerve signal, calcium ion channels open and the increase in intracellular calcium induces the vesicles to fuse to the cell membrane (Patrick 2001). This fusion releases acetylcholine into the synaptic cleft and stimulates the opening of sodium and potassium channels in the postsynaptic neurons in the brain, heart, lungs and skeletal muscle tissues (Patrick 2001). AChE works in the neuronal synapse to terminate the signal by hydrolysis of acetylcholine to form inactive acetate and choline (Patrick 2001). Choline is recycled back into the presynaptic cleft by a membrane transport protein and the cycle continues (Patrick 2001). Other CNS neurotransmitters also act to regulate acetylcholine (Patrick 2001). Norepinephrine suppresses cholinergic activity and nitric oxide promotes acetylcholine release (Patrick 2001). Cotransmitters are released with acetylcholine and these act to mediate the effects of acetylcholine by producing a longer lasting effect or to change an effect, depending on the circumstances (Patrick 2001).

Measurement of exposure to OPs is based on erythrocyte cholinesterase often called acetylcholinesterase (AChE) or true cholinesterase (Coye et al. 1987). Plasma cholinesterase has been called pseudocholinesterase, butyrylcholinesterase (BuChE) and benzoylcholinesterase. BuChE is a liver protein found in plasma, heart and brain, but its physiological function remains unknown, probably due to the fact that butyrylcholine is not a physiological substrate in mammalian brain but is used only to distinguish BuChE from AChE (Wright et al. 1993). BuChE appears to have a regulatory effect on AChE and serves as a buffering system to lower anticholinesterase compounds in the neuromuscular junction (Rotundo et al. 1994). AChE is a better measure of acute toxicity

than BuChE because it is found in brain, skeletal muscle, peripheral tissues, and red blood cells, and its function is clearly understood.

Biochemistry of the neurotransmitter acetylcholine

Acetylcholine, the physiological substrate for AChE, is a flexible molecule and can take on nine stable conformations due to bond rotation along the length of its chain (Crosby 1998). In one of these conformations, the nitrogen group swings around forming a circular molecule and comes into close proximity with the carbonyl group on the acetoxy end of the molecule (Crosby 1998). Acetylcholine is vulnerable to hydrolysis because the quarternary nitrogen with its positive charge has an electron-withdrawing effect on the carbonyl oxygen increasing its electronegativity and making it prone to a nucleophilic attack (Crosby 1998).

The serine hydrolases are a large class of enzymes that can hydrolyze ester, peptide and amide bonds (Timbrell 2000). The general mechanism is a nucleophilic attack by a serine in the active site of the enzyme that attacks the carbonyl carbon of the substrate and forms an acyl-enzyme intermediate, which is hydrolyzed to produce the products (Patrick 2001; Timbrell 2000). AChE is a member of the class of serine esterases allowing it to break an ester bond using hydrolysis (Patrick 2001). Structurally it resembles a tree with three branches (Patrick 2001). The trunk is a collagen molecule that tethers the enzyme to the cell membrane (Patrick 2001). The three branches are made up of disulfide bridges that hold the enzyme above the membrane (Patrick 2001). The enzyme has four submits each of which contains an active site resulting in a total of 12 active sites per enzyme (Patrick 2001).

The mechanisms by which acetylcholine or its analogs bind to AChE have been characterized. The binding pocket consists of an anionic binding region and an ester binding region (Patrick 2001; Timbrell 2000). In the anionic binding region, it is thought that the oxygen on an asparagine side chain forms an ionic bond with the quarternary nitrogen group of acetylcholine (Patrick 2001; Timbrell 2000). In the ester binding region, where catalysis occurs, a tyrosine forms a hydrogen bond with the ester oxygen on acetylcholine and glutamate, histidine and serine are involved in actual hydrolysis of the ester (Patrick 2001; Timbrell 2000). Catalysis is initiated by an acid/base reaction by histidine followed by a nucleophilic attack by serine (Patrick 2001; Timbrell 2000). Hydrogen bonding between the carboxyl group of glutamate and a nitrogen (N-1) of the histidine imidazole ring enhances the ability of the nitrogen in the three position of the histidine ring to act as a base and abstract a proton from the serine hydroxyl group on the enzyme (Patrick 2001). This cooperation between histidine and serine makes the serine oxygen into a better nucleophile allowing it to readily attack the carbonyl carbon of acetylcholine (Patrick 2001). This reaction produces a tetrahedral intermediate that is more stable than acetylcholine, most likely due to stabilization by hydrogen bonding in an oxyanion hole (Patrick 2001). The stabilization of the intermediate relative to the substrate is what makes the reaction possible (Patrick 2001). The tetrahedral intermediate collapses and releases choline, leaving behind an acyl enzyme (Patrick 2001). The acyl enzyme is attacked by a water molecule and, in a hydrolysis reaction, releases the acetate (Patrick 2001). The enzyme is ready for another cycle.

Genetic susceptibility to organophosphorus compound toxicity

A number of genetic polymorphisms have been characterized that increase susceptibility to certain chemicals and diseases (Nakajima et al. 2000). Those in the initial steps of detoxification include certain CYP genes in the cytochrome P450 family of enzymes, alcohol dehydrogenase and aldehyde dehydrogenase (Nakajima et al. 2000). Those involved in conjugation reactions, which increase polarity and allow for excretion, include UDP-glucuronyltransferase, glutathione S-transferase, N-acetyltransferase and NAD(P)H-quinone oxidoreductase (Nakajima et al. 2000). These have been found to detoxify carcinogens, such as polycyclic aromatic hydrocarbons, arylamine nitrosamines, aflatoxin B1, benzene and metabolites and benzo[a]pyrene epoxides (Nakajima et al. 2000). The P-450 monooxygenases and flavin monooxygenases are involved in the desulfuration step that creates the OP oxon (Levi et al. 1992). Paraoxonase has been shown to be an important early step in the detoxification of the oxons of chlorpyrifos and diazinon created in the Phase I reactions (Costa et al. 2003). Polymorphisms in the paraoxonase gene have been linked to genetic susceptibility to OP toxicity (Costa et al. 2003). Additional work needs to be done on the contribution of CYP polymorphisms to OP toxicity (Costa 2001).

The paraoxonase-1 (PON1) gene is a member of a multigene family containing PON1, PON2 and PON3 (Primo-Parmo et al. 1996). The enzyme product of this gene is an apolipoprotein associated with high-density lipoprotein (HDL) particles whose biological function is to keep low-density lipoproteins (LDL) in a reduced state (Geldmacher-von Mallinckrodt et al. 1988). It has been studied more due to its role in

OP metabolism than due to its biological role in lipid metabolism (Costa et al. 2003). Strong evidence exists that PON1 is important in detoxifying xenobiotics (Costa et al. 2003). First, when injected into rats or mice, it protects against toxicity from diazoxon, chlorpyrifos oxon, and soman and sarin oxons (Li et al. 1995). Second, the catalytic efficiency of substrate hydrolysis corresponds to sensitivity to these OPs (Furlong et al. 1989; Li et al. 2000). Third, PON1 knockout mice are sensitive to OP toxicity after administration of oxon forming OPs (Li et al. 2000; Shih et al. 1998). Although the PON1 gene was named after parathion and its oxon derivative paraoxon and has great activity *in vitro* towards paraoxon, PON1 knockout mice do not show increased sensitivity to paraoxon, although they were extremely sensitive to the chlorpyrifos oxon and diazoxon (Costa et al. 2003; Li et al. 2000). Paraoxon does not appear to metabolize paraoxon efficiently *in vivo* and CYP enzymes or carboxylesterases are involved in detoxifying paraoxon (Pond et al. 1995).

Paraoxonase activity has been characterized in only a few OP and nerve gas compounds. It is now believed that paraoxon is not a physiological substrate for paraoxonase, but the OPs diazinon, chlorpyrifos, which produce very toxic oxons, and the nerve gases sarin and soman are well-established substrates (Costa et al. 2003; Furlong et al. 1989; Geldmacher-von Mallinckrodt et al. 1988). Currently the best markers of PON1 genetic susceptibility are the –108 polymorphism in the promoter region of the PON1 gene associated with expression levels (Brophy et al. 2001) and the glutamine to arginine mutation at position 192 in the paraoxonase protein resulting in substrate-dependent enzyme activity (Costa et al. 2003). The –108 polymorphism contributes 22.4% to the variation in PON1 expression levels (Brophy et al. 2001).

Polymorphisms in the PON1 gene were first linked to ill health in sheep dippers because it is the key enzyme involved in hydrolyzing diazoxon, the active metabolite of the OP diazinon, often used in sheep dip (Cherry et al. 2002). A case-control study of ill health in sheep dippers showed that the odds ratios for the polymorphism at position 192 (a glutamine to arginine substitution) in those with at least one polymorphic allele was 1.93 (95% confidence interval 1.24 to 3.01) (Cherry et al. 2002). Subsequently, two additional polymorphisms in the promoter region of the PON1 gene have been identified at the –909 and -162 positions (Brophy et al. 2001; Chen et al. 2003). The identification of promoter polymorphisms requires that both expression levels and activity of the enzyme should be measured to fully characterize PON1 status. Brophy et al. (2001) performed expression assays from deletion mutants of the PON1 promoter region and identified the same three promoter polymorphisms (Brophy et al. 2001). No other polymorphisms were identified from 11,506 base pairs upstream of the initiation site of transcription (Brophy et al. 2001).

Potential targets for organophosphorus compounds resulting in delayed neurotoxicity

In addition to the accepted short-term effects of AChE and BuChE inhibition, an intermediate syndrome, which can develop between 24 and 96 hours after exposure, has been identified (Senanayake et al. 1987). These intermediate symptoms include cranial nerve palsies, neck and proximal limb weakness and respiratory paralysis and these symptoms do not respond to atropine or pralidoxime, indicating that AChE is not involved (Leon-S et al. 1996). OPIDN or simply delayed neuropathy results in axonopathy which appears anywhere from about eight days to three weeks following absorption of an effective dose of an OPIDN-producing compound (Abou-Donia et al.

1990; Abou-Donia 1981). Symptoms include tingling and burning sensations in the limb extremities followed by weakness in the lower limbs resulting from the longest and largest nerve fiber tracts in the central and peripheral nervous systems degenerating (Richardson 1992). In severe cases, paralysis can result (Richardson 1998).

The theories behind the role of neuropathy target esterase (NTE) go back to about 1970 when researchers were studying molecular changes associated with OP toxicity (Johnson 1969). NTE knockout mice die after embryonic day eight suggesting that it is required for neuronal development (Winrow et al. 2003). The cause of death of the mice with the disrupted NTE gene was not apparent, but the researchers speculated that it might be a neural tube defect (Winrow et al. 2003). In general, rodents have not been considered the ideal animal model of OPIDN because they do not develop hind-limb paralysis after exposure to OP compounds (Abou-Donia 1981). Hens have been identified as the ideal animal model because of their susceptibility to ataxia, as seen in human OPIDN (Abou-Donia 1981; Randall et al. 1997).

The hypothesized mechanism for OPIDN involves a two-stage process where NTE is progressively inhibited by certain phosphates, phosphonates and phosphoroamidates. Inhibition is the result of the active site serine of NTE becoming phosphorylated, as in acetylcholinesterase inhibition. Neuropathic effects are not observed until approximately 70 to 90% of the enzyme is inhibited (Veronesi et al. 1992). The inhibition is followed by a second step called "aging" (Lotti et al. 1993). Aging involves the loss of an alkoxy or aryl group, leaving a negatively charged phosphate in the active site (Richardson 1992). Once aging has occurred, NTE cannot be reactivated (Richardson 1992). Neuropathic OPs cause aging after inhibition and some OPs protect

against aging if administered prior to the neuropathic OP (Veronesi et al. 1992). To complicate matters further, some carbamates, phosphinates, and sulfonyl halides that have been shown to provide protection from OPIDN appear to require inhibition of NTE (Veronesi et al. 1992). It was thought that the ability to age the inhibited NTE both was predictive of the occurrence of adverse neuropathic effects and was dependent on the extent of inhibition, but exceptions to this theory have been shown (Lotti et al. 1993). Some phosphoroamidates that cause OPIDN form an inhibited NTE that does not age, using NTE reactivity with potassium fluoride as a test of aging (Lotti et al. 1993). Further, repeated high doses of the protective compounds cause a mild neuropathy that resembles OPIDN (Lotti et al. 1993). Some OPs that inhibit NTE as much as 75% and proceed to age the NTE enzyme do not cause OPIDN, except at high doses (Lotti et al. 1993). Although there appears, for the most part, to be an inverse relationship between protection by an OP and initiation of NTE aging, there is some evidence of interactions between OPs (Lotti et al. 1993). Dichlorvos, a commonly applied OP in agriculture, is an initiator of NTE aging and lacks any protective effect at any dose, but aging may not be the sole criteria for OPIDN (Lotti et al. 1993). OPIDN might result from the aged phosphorylated product causing a gain of function and not merely the loss of NTE activity (Atkins et al. 2000).

The mouse model showed that NTE levels were high throughout the brain, particularly in the cortex and the Purkinje cells of the cerebellum (Winrow et al. 2003). Both NTE protein levels and activity were lower in the heterozygous mutant mice (Winrow et al. 2003). No differences were seen in AChE activity between wild type and mutant mice (Winrow et al. 2003). At doses generating greater than 85% inhibition of

NTE when exposed to the OP ethyl octylphosphonofluoridate (EOPF), the mutant mice showed greater sensitivity and higher mortality (Winrow et al. 2003). Mutant and wild-type mice were administered EOPF at 10% of that causing mortality and the total distance the mice traveled and their vertical behavior were recorded for a ten-day interval. The mutant mice showed signs of hyperactivity compared to the wild-type mice (Winrow et al. 2003).

The physiological role of NTE has recently been determined to be a lysophospholipase, which degrades phosphatidylcholine to glycerophosphocholine (Quistad et al. 2004; Quistad et al. 2003; Zaccheo et al. 2004). The levels of phosphatidylcholine or lecithin, a major membrane lipid, are tightly regulated (Wang et al. 1999). Experiments from the Glynn laboratory showed that the catalytic domain of NTE deacylates the sn-2 position of the fatty acid slowly, but rapidly deacylates the resulting lysophospholipid (Zaccheo et al. 2004). How NTE and other lysophospholipases regulate lysolecithin is mostly unknown (Wang et al. 1999), but what is known is that increased lysophospholipid levels are associated with a number of diseases including hyperlipidemia, inflammation, lethal dysrhythmias in myocardial ischemia and segmental demyelination of peripheral nerves (Wang et al. 1999). Lysophospholipids have been shown to activate G-protein coupled receptors and to mediate signal transduction (Wang et al. 1999). Low levels of lysophospholipase activity in brain would result in elevated lysolecithin levels (Quistad et al. 2004). High levels of lysolecithin cause demyelination of axons in the central and peripheral nervous systems, resulting in reduction of neural transmission (Jean et al. 2002).

Quistad and Casida (2004) showed that dichlorvos and chlorpyrifos oxons were moderately potent inhibitors of total lysophospholipase, but paraoxon was inactive towards lysophospholipases. Two fluorophosphonates were more potent inhibitors of total NTE-lysophospholipase, reflecting the greater toxicity of the nerve gas agents compared to the OP pesticides (Quistad et al. 2004).

NTE is highly expressed in cerebral cortical areas of hen, rat and human brain (Richardson 1992). Non-neural tissues where it is found include placenta, spleen, lymphocytes and testis (Richardson 1992). NTE is only one of greater than 100 lipases that may be targets of OP compounds (Bracey et al. 2002). As a group, these lysophospholipases have been shown to activate protein kinases, including PKC, MAPK, Raf-1 kinase and c-Jun N-terminal kinase and to stimulate DNA binding activity of activator protein 1 and nuclear factor kappa B (Wang et al. 1999). Imbalances in phospholipid synthesis and degradation pathways leading to dysregulation and changes in available phosphatidylcholine may lead to changes in signaling pathways that become imprinted over time, resulting in changes in neurotransmission pathways.

DFP, a potent inhibitor of NTE and other lysophospholipases, was the chemical used to produce the Flinders Sensitive Line (FSL) of rats that are sensitive to OP toxicity and are regarded as an excellent model of depression in humans (Overstreet et al. 1996; Overstreet et al. 1982; Overstreet 1993). These selectively bred, OP-sensitive rats show both cholinergic supersensitivity and many of the same symptoms reported in humans with multiple chemical sensitivity (Overstreet et al. 1996). Upon exposure to stressors, the FSL rats show psychomotor retardation symptoms and are impaired in active avoidance paradigms compared to the Flinders Resistant Line (FRL) rats that are bred to

be resistant to OP toxicity (Overstreet et al. 1990). These studies suggest a link between OP compounds, choline metabolism and depression if, in fact, the FSL rats do represent a human model of depression.

Epidemiology of depression

Epidemiological studies have identified prior depressive episodes, a family history of major depressive disorder, prior suicide attempts, lack of social support, medical comorbidity, stressful life events, current substance abuse and being female as risk factors for depression (Katon 2003; Kornstein 1997; Shafffery et al. 2003). Results from the National Comorbidity Study found that the lifetime prevalence of depression in women in the United States is 21.3% and in men, 12.7% (Kessler et al. 1994). Psychiatric epidemiological studies in communities, rather than in the clinical setting, have examined patterns of depressive symptoms over time and found that individuals fall into one of three categories: consistently asymptomatic, consistently symptomatic and both asymptomatic and symptomatic (Aneshensel 1985; Lin et al. 1984). These categories were simplified into the mover-stayer model of depression (Aneshensel 1985). The movers transition between symptoms and no symptoms, and the stayers stay symptomatic (Aneshensel 1985). The *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV) classifies depression without mania into chronic, minor depression (dysthymic disorder) and major depressive disorder (MDD) (APA 1994). Dysthymic individuals are the movers and MDD individuals may be movers or stayers by the DSM-IV definition (APA 1994).

Dysthymic depression is chronic depression of at least two years duration with depressed mood for most of the day and for more days than not (APA 1994). Criteria for

dysthymic depression requires having at least two of the following when depressed mood is present: (1) poor appetite or overeating; (2) insomnia or hypersomnia; (3) low energy or fatigue; (4) low self-esteem; (5) poor concentration or difficulty making decisions; and (6) feelings of hopelessness (APA 1994). A diagnosis of dysthymic disorder requires that an individual have a depressed mood and other symptoms for at least two months during a two-year period. It can result from an incompletely remitted or a poorly treated major depressive episode and can be recurrent. It can also include bouts of MDD.

The criteria for MDD is having at least one episode with at least five of the following symptoms: (1) depressed mood; (2) markedly diminished interest or pleasure in most activities; (3) significant weight loss or weight gain; (4) insomnia or hypersomnia with psychomotor agitation or retardation; (5) fatigue or loss of energy, feelings of worthlessness, diminished ability to think or concentrate; and (6) recurrent thoughts of death (APA 1994). These symptoms must persist most of the day, nearly every day, for at least two weeks to meet the MDD definition (APA 1994).

There are presently 18 distinct animal models of depression, but four are thought to be the best models of human depression due to having high validity (Yadid et al. 2000). Validity is based on how well the animal models express human depression characteristics, whether they are derived in a manner that parallels our understanding of MDD, and whether they respond to antidepressants in a manner similar to human depressives (Willner 1984). Most of these models are based on stressing animals at some early point in their lives using inescapable shock, early Rapid Eye Movement (REM) sleep deprivation, and drugs, all which can promote depressive-like behavior in animal adulthood (Shafffery et al. 2003). These models include learned helplessness, chronic

mild stress, social defeat, maternal separation, and the Flinders Sensitive Line (FSL) rats (Shafffery et al. 2003).

Biological basis for depression

Depression is an illness with a basis in environmental stress and alterations in neurotransmitter systems including the noradrenergic, cholinergic, serotonergic and dopaminergic pathways (Janowsky et al. 1987; Stone et al. 2003). One of the earliest observations was that monoamine depletion, which reduces the available serotonin or norepinephrine neurotransmitters, results in depressive symptoms (Bunney et al. 1965; Schildkraut 1965). This observation was supported by the efficacy of the monoamine oxidase inhibitors and tricyclic antidepressants used to treat depression and by the determination that these drugs increase monoamine receptor sensitivity (Charney et al. 1981). Recent research suggests that epinephrine acting at alpha-1 adrenoceptors may also play a role due to its stress-mediating effects (Stone et al. 2003), and is consistent with a previous finding that epinephrine effects are reduced in response to physostigmine in depressed patients (Janowsky et al. 1986). The most accepted hypothesis is that no single neurotransmitter is responsible for depression, but that many alterations in interacting pathways cause the dysregulation that leads to depression (Siever et al. 1985). The understanding of the biological mechanisms and neurotransmitters systems underlying depression can be credited to the study of drug effects in animal models and psychiatric patients (Janowsky et al. 1972).

Cholinomimetics are drugs that mimic the effects of acetylcholine (Janowsky et al. 1987). The cholinergic effects can be achieved by either administration of cholinergic agonists, which are primarily muscarinic agonists such as arecoline, oxotremorine,

pilocarpine, scopolamine, and RS-86 or cholinesterase inhibitors such as DFP, physostigmine and OP compounds that inhibit acetylcholinesterase (Janowsky et al. 1987; Overstreet et al. 1996). Cholinergic agonists act by increasing activity of muscarinic acetylcholine receptors resulting in the same effect as inhibiting acetylcholinesterase, both resulting in increased acetylcholine activity.

Researchers over the past 30 years have proposed that mood disorders stem from an imbalance between adrenergic and cholinergic factors (Figure 4). Janowsky and colleagues published the cholinergic-adrenergic hypothesis of depression and mania in 1972 (Janowsky et al. 1972) and provided evidence for the hypothesis using individuals exposed to physostigmine and methylphenidate (Janowsky et al. 1973). The central premise of the hypothesis is that depression is a consequence of central cholinergic predominance and mania is a consequence of adrenergic predominance. Anticholinergic drugs have been shown to induce depression and to reduce mania in bipolar patients (Janowsky et al. 1987). The most studied anticholinesterases used to induce depression or reduce mania are DFP and physostigmine (Janowsky et al. 1973). Research participants who were given OPs in the 1970s produced a short-term response that included retarded depression, fatigue, irritability, impaired concentration, confusion and anxiety (Janowsky et al. 1972; Janowsky et al. 1973). Apparently, the administration of certain drugs can alter the cholinergic balance and induce the appearance of depression or, in some cases the adrenergic balance, and induce mania (Janowsky et al. 1987; Janowsky et al. 1994).

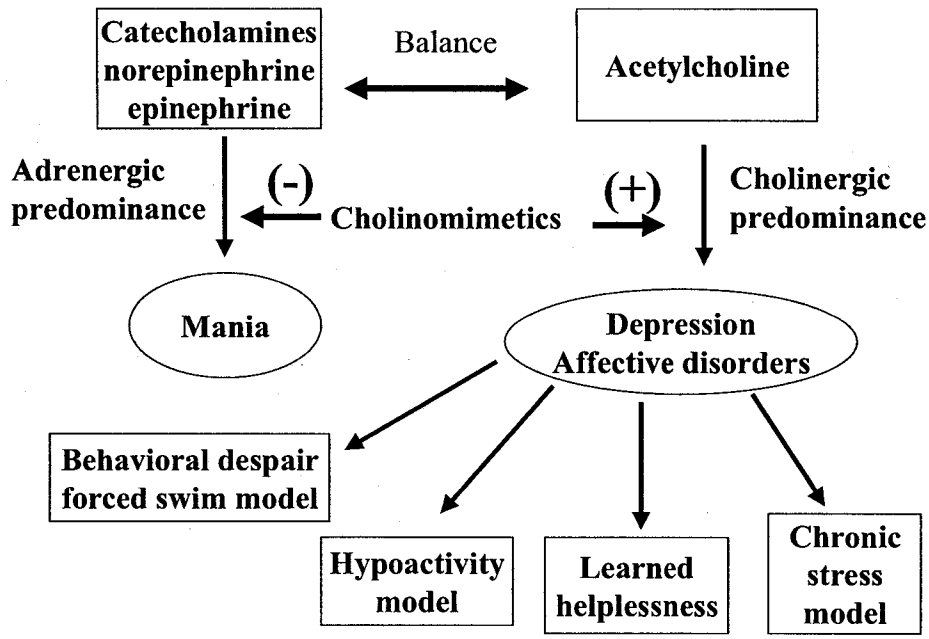


Figure 4. The Janowsky, et al. (1972) Balance Hypothesis.

A complex web of factors, including corticotropin-releasing hormone, hypothalamic-pituitary-adrenal (HPA) hormones and cytokines mediate neurotransmitter networks (Stone et al. 2003). Impairment of the system during stress and depression involves changes in epinephrine binding at noradrenergic receptors and changes in adrenocorticotropic hormone (ACTH), corticosterone and cytokines (Shafffery et al. 2003; Stone et al. 2003). The loss of positive, motivated behavior is thought to be due to a reduction of central dopaminergic neurotransmission in basal ganglia (Stone et al. 2003). Decreased levels of dopaminergic metabolites are found in the cerebral spinal fluid of depressed individuals (Stone et al. 2003). The totality of these observations suggests that many neurotransmitter systems influence mood.

In addition to the observations that cholinergic agonists and cholinesterase inhibitors reduce mania in bipolar patients, rat studies show that increased activity in the

cholinergic system results in a later compensatory antagonistic activation of the adrenergic system (Janowsky et al. 1987). The observed withdrawal effects from antidepressants resulting in a depressed mood, anxiety, withdrawal, agitation and insomnia, are believed to be associated with muscarinic receptor hypersensitivity or what has been termed "cholinergic overdrive" (Dilsaver et al. 1984). Additionally, reciprocal interactions have been observed between agents that increase catecholaminergic activity and those that increase cholinergic activity (Janowsky et al. 1987). Methylphenidate reverses psychomotor retardation, which occurs after anticholinesterase agents (Janowsky et al. 1994). Psychomotor retardation in humans is associated with feelings of fatigue, lack of thoughts, and anergy with decreased activity (Janowsky et al. 1994). Methylplenidate antagonized by physostigmine and amphetamine is antagonized by arecoline (Janowsky et al. 1987).

Evidence specifically implicating acetylcholine in depression can be found in the literature. Abnormal levels of cortical choline have been reported in several brain imaging studies of depressed individuals (Charles et al. 1993; Steingard et al. 2000). After treatment with antidepressants, these increased levels reverted to normal levels (Charles et al. 1993). The acetylcholine precursors choline and lecithin can induce depression (Casey 1979; Davis et al. 1979; Tamminga et al. 1976).

Patients with affective disorders are more sensitive to the negative affect and inhibitory effects of cholinomimetics than are controls (Janowsky et al. 1980; Janowsky et al. 1981). Using the Profile of Mood States Scales, self-reported anxiety, depression, hostility, confusion and decreases in elation were significantly greater in affective

disorder patients than in other psychiatric patient groups or normal controls after physostigmine infusion (Janowsky et al. 1980; Janowsky et al. 1981).

Physostigmine and other cholinomimetic drugs cause increases in HPA axis activity and result in increased cortisol secretion and elevated ACTH levels, characteristic of depression in humans (Risch et al. 1981). Increased beta endorphin, released following stimulation by ACTH and corticotropin-releasing hormone (CRF), is also observed in depressed patients, as well as those administered physostigmine and arecoline (Risch et al. 1981)

Some of the most interesting effects of cholinomimetics are on the sleep parameters (Janowsky et al. 1987; Poland et al. 1997). Sleep abnormalities are related to dysregulation of the muscarinic cholinergic system (Gillin et al. 1982; Sitaram et al. 1982). Sleep EEG abnormalities in depression in humans is well studied and is associated with decreased REM latency where the first REM sleep cycle occurs earlier in the depressed (65 minutes) than in nondepressed (90 minutes) and increased REM density where there are more REM sleep events (Shafffery et al. 2003). In addition, REM sleep time is increased in depressed compared to nondepressed and wave patterns are altered indicating different types of brain activity during the sleep cycle (Shafffery et al. 2003). Cholinergic agonists administered to nondepressed individuals cause the same effects as those seen in depressed individuals (Janowsky et al. 1987; Poland et al. 1997). Arecoline, a cholinergic agonist, was shown to significantly shorten REM latency when infused into patients with an affective disorder or with a family history of affective disorder compared to normal controls (Janowsky et al. 1987). Berger et al. (1983) found that physostigmine induced arousal and awakening from sleep more frequently in patients

with affective disorders than in normal controls (Berger et al. 1983) and later showed supershortening of REM latency in depressives orally administered the muscarinic agonist, RS-86 (Berger et al. 1985).

Presently there are four models of mood disorders (Janowsky et al. 1987). The first is the monoamine-acetylcholine interaction model based on evidence showing that psychostimulants, which increase catecholaminergic activity, show reciprocal effects with cholinomimetics, which increase cholinergic activity (Janowsky et al. 1987). The second model is that the cholinergic system acts alone or with other neurotransmitter systems to directly regulate mood (Janowsky et al. 1987). The third model (The Balance Hypothesis) states that a pharmacologically-induced change in acetylcholine level causes changes in systems other than the cholinergic system, such as GABA, serotonin, dopamine and norepinephrine, but does not directly cause depression (Janowsky et al. 1987). The fourth model asserts that acetylcholine acts as a regulator of stress, and depression is one possible response to the stress (Janowsky et al. 1987). In support of the fourth hypothesis, acetylcholine turnover increases with increases in stress in animals, and exaggerated responses are seen in rats that are bred to be cholinergically supersensitive (Janowsky et al. 1987).

Janowsky's adrenergic-cholinergic imbalance model fits the physiological, behavioral and neurobiological data better than other hypotheses in humans or animals (Shafffery et al. 2003). All of the models reduce to two current theories: (1) that acetylcholine alters other neurotransmission systems (GABA, serotonin, dopamine or norepinephrine) causing depression but does not directly cause depression, and/or (2) that acetylcholine regulates the effects of stress and depression may be just one possible

outcome. All models and theories to date include the importance of the cholinergic system, but knowing what role it plays in relation to other neurotransmission systems will be difficult to tease out. The FSL rat model has proven beneficial to our present understanding of depression and changes in neurotransmission systems.

Developed at the University of Flinders in Australia, the FSL rat represents the genetic predisposition for supersensitivity to cholinergic agonists and is the model used for understanding human genetic predisposition to depression (Overstreet et al. 1982). These rats are supersensitive to cholinergic and serotonergic (5-HT1A) agonists and show characteristic symptoms of depression found in humans (Overstreet et al. 1998). They have increased rapid-eye movement (REM) sleep, appetite and weight changes and reduced activity, and increased anhedonia, all of which can be reversed with tricyclic antidepressants and serotonin reuptake inhibitors (Overstreet et al. 1982). The biochemical similarities with humans are an HPA axis dysfunction, abnormalities in slow-wave and REM sleep, and immune system dysfunction (Yadid et al. 2000). The FSL rats exhibit exaggerated immobility in the forced swim test compared to the FRL control rat (Overstreet et al. 1998). FSL rats also have increased hippocampal and striatal muscarinic acetylcholine receptors, indicating an upregulation of the muscarinic noradrenergic pathway. This observation supports the Janowsky balance hypothesis that increases in cholinergic activity may lead to increases in the adrenergic pathways, which involve the catecholamine neurotransmitters.

Conclusion

OPs are a large and diverse group of compounds with varying degrees of specificity, activity, and toxicity. Dose cannot easily be predicted from exposure due to

formulation differences in the compound, the mixing and application methods used, personal protective equipment worn, and individual biological differences in detoxification, metabolism and excretion. Although acute health effects are well-defined, chronic health effects after exposure can be vague, for example, sheep-dippers that report having ill health from OP pesticide exposure. Beginning with the studies of Grob and Dille, depression was reported as an acute effect. This is most likely due to stimulation of cholinergic pathways, and, possibly a compensatory mechanism of upregulation of muscarinic pathways, as shown by Janowsky and others. Depression was also reported as a chronic outcome of pesticide exposure and may result from inhibition and/or aging of NTE or other critical lipases with subsequent alterations in phospholipid metabolism and G-protein receptor signaling.

The literature suggests two possible physiological responses to pesticide exposure (Figure 5). First, tolerance has been shown to occur in mice and rats administered subacute doses of OP compounds, including OP pesticides. Examining the brains of these animals showed decreased responsiveness to muscarinic or nicotinic receptors, as an adaptation to chronically elevated acetylcholine. This observation was confirmed in FRL rats that showed subsensitivity to muscarinic agonists. Reduced muscarinic activity may be due to either a decrease in receptor density or attenuated receptor binding affinity to muscarinic agonists. Some studies show reduced choline uptake in response to elevated acetylcholine. The second possible response to pesticide exposure is development of sensitivity where symptoms occur with subsequent exposure to OPs and carbamates, which might occur in those who have been poisoned or are genetically susceptible to pesticide poisoning. Sensitization may or may not be related to effects on

other enzyme systems, such as NTE, and may or may not be related to multiple chemical sensitivity syndrome.

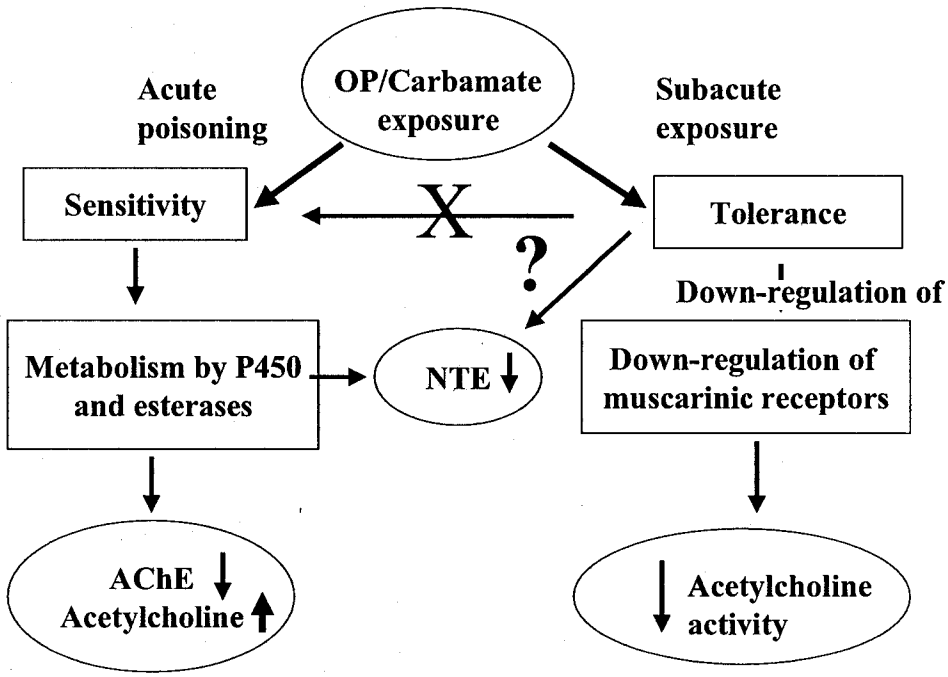


Figure 5: Model of acute and subacute pesticide exposures and effects on acetylcholine activity.

NTE and other lipases that regulate phospholipid signaling molecules and availability of phospholipid precursors may be related to the long-term neuropsychiatric effects seen in those who have had a high-level pesticide exposure. Increased lysolecithin in the cell may lead to an increase in cholinergic activity and changes in crosstalk between adrenergic and serotonergic pathways, as well as alterations in GABA and dopamine systems. Lysophospholipases are involved in G-protein signaling, which is also critical in signaling by catecholamines, including norepinephrine and epinephrine. Clearly, there is the possibility that NTE and other lipase targets of OP compounds may alter neurotransmission pathways. Further work should address NTE inhibition and aging on downstream effectors.

Most useful to understanding the connection between OP pesticides and the neurotransmission systems associated with depression has been the FSL and FRL rats developed for sensitivity to DFP and resistance to DFP, respectively. It is informative that the FSL rat has been shown as a valid and useful model in human depression and that they are supersensitive to cholinergic and serotonergic agonists. These rats have allowed insights into the connections between the cholinergic and adrenergic systems, and also into cholinergic and serotonergic systems. Although cholinergic sensitivity has not yet been shown to be a strong marker of susceptibility to affective disorders, there is good evidence that being susceptible to affective disorders increases sensitivity to cholinergic agonists. This means that individuals with a personal or family history of affective disorders who are later exposed to pesticides at high enough levels to overcome their detoxification systems, may be at an increased risk of developing a more severe mood disorder than those without the genetic susceptibility. Additionally, those with both compromised detoxification systems and a genetic predisposition to affective disorders may be at the highest risk of a mood disorder or psychotic episode after an even moderately high exposure to an OP pesticide (Figure 6).

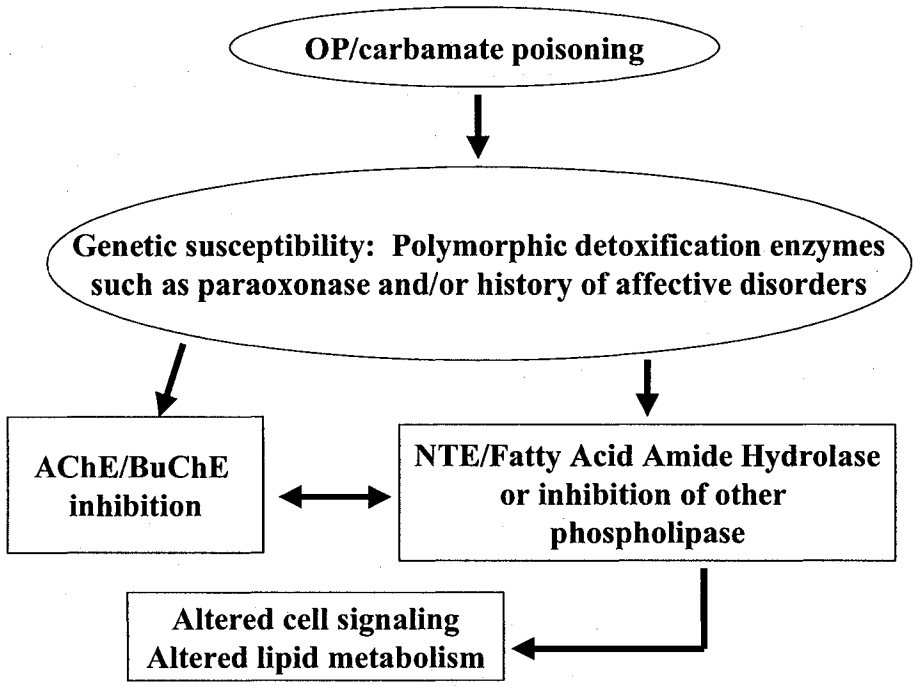


Figure 6. Hypothesized pathway of pesticide poisoning and chronic health effects.

The addition of stress to the physiological system results in increased acetylcholine turnover and an increased release of the neurohormones epinephrine, ACTH, cortisol, ß-endorphin, prolactin, growth hormone and vasopressin, and may result in a severe affective disorder. Such symptoms have been reported to be anxiety, depression, fatigue and hostility. Depression may be just one mood disorder that results from OP exposure, and the expression of the mood disorder may depend greatly on the genetics underlying a number of neurotransmission systems in an individual.

CHAPTER TWO: DIAGNOSED DEPRESSION AND LOW, INTERMEDIATE, AND HIGH PESTICIDE EXPOSURES IN SPOUSES OF IOWA AND NORTH CAROLINA FARMER PESTICIDE APPLICATORS IN THE AGRICULTURAL HEALTH STUDY

BACKGROUND

Studies over the past forty years show evidence of an association between neurological effects and exposure to organophosphorus (OP) ester insecticides (Dille et al. 1964; Ray et al. 2001). In the past decade, several studies have observed an association between pesticide exposure and depressive symptoms, often observed in those with very high exposures causing acute poisonings (Amr et al. 1997; Reidy et al. 1992; Savage et al. 1988; Stallones et al. 2002). High level exposure or acute toxicity is defined as exposure producing a dose sufficient to cause clinically detectable acetylcholinesterase inhibition, resulting in the classical symptoms of miosis, headache, nausea, dizziness, fasciculations, vomiting, abdominal cramps, diarrhea, sweating and lacrimation (Brown et al. 1998; Gunderson et al. 1992). Low-level exposures are defined as exposures that do not induce symptoms associated with a detectable cholinergic response (Brown et al. 1998). Some researchers argue that low-dose, long-term exposure can produce neuropsychological changes (Salvi et al. 2003; Stephens et al. 1995), while others have suggested that the association requires a high exposure or a poisoning (Ames et al. 1995; Daniell et al. 1992; Fielder et al. 1997; Rodnitzky et al. 1975; Rosenstock et al. 1991). Because many of the individuals who are studied after having an acute poisoning have worked as pesticide applicators for many years, long-term, low-dose exposure may act as a confounder in studies of high exposure or acute poisoning (Rosenstock et al. 1991; Savage et al. 1988; Steenland et al. 2000). Additionally, in studying long-term, low-dose

exposure, intermediate level exposures may or may not induce a cholinergic response. Adaptive mechanisms may produce tolerance to OPs causing a down-regulation of both acetylcholine release and receptor sensitivity resulting in a high exposure that is not recognized as a substantial exposure (Schwab et al. 1981).

Whether low-dose, long-term exposure or a pesticide poisoning predisposes an individual to depression or anxiety and what the biological mechanisms for long-term effects might be remains under investigation. Assuming subtle effects occur in the absence of acetylcholinesterase inhibition, other mechanisms are needed to explain the intermediate and long-term neurological effects of exposure (Ray et al. 2001). Animal studies show that carboxylesterases in the central nervous system are more sensitive to OPs than acetylcholinesterases (Chanda et al. 1997; Chemnitius et al. 1983). There is evidence that certain OPs may target neuropeptide metabolism in the central nervous system (O'Neill 1981; Richards et al. 2000). Future work will most likely elucidate other targets for OPs given the tremendous number of hydrolase and esterase enzymes in biological systems.

The association of depression and anxiety to pesticide exposure has been demonstrated primarily in studies of men, as well as in a few studies of both men and women, but these studies have suffered from small numbers and limited power (Reidy et al. 1992; Savage et al. 1988; Stephens et al. 1995). Savage et al. (1988) studied 100 acutely poisoned individuals matched to 100 non-exposed controls, Reidy et al. (1992) studied only 21 exposed individuals and 11 controls and Stephens et al. (1995) studied 146 sheep dippers matched to 143 quarry workers. All of these studies involved different chemical exposures and, specifically, different OP exposures over varying lengths of

time. The severity of clinical effects of OP exposure is due to the rate at which phosphorylation of a target biological enzyme occurs, and this can vary greatly depending upon the chemical structure of the OP and the enzyme target (Dille et al. 1964; Karalliedde et al. 2003). Whether the effects are solely due to exposure to OPs, or to herbicides, fungicides, fumigants, or solvent exposure, or a combination of these, is difficult to determine because pesticide applicators apply a variety of compounds. Neurological effects have been reported to be associated with solvents (Spurgeon et al. 1992), phenoxy herbicides (Green 1991), and fungicides (Baldi et al. 2001). Using death certificate data from 1988 through 1992, van Wijngaarden (2003) compared occupations with potential exposure to pesticides to those unlikely to be exposed to pesticides and observed a significantly elevated risk of mortality due to psychoses and neurotic disorders associated with pesticide exposed occupations (van Wijngaarden 2003).

Pesticide exposure varies by country, occupational setting and safety behavior. Studies of pesticide applicators in developing countries have shown higher exposures than in developed countries (Jeyaraatnam 1990; Forget 1991). Occupational exposures differ by whether someone works as a formulator in a chemical manufacturing plant, as a commercial pesticide applicator, or as a farmer pesticide applicator. Commercial formulators are exposed to various pesticides forty hours per week for years at a time (Amr et al. 1997). In the Agricultural Health Study (AHS), farmer applicators applied pesticides an average of 16 years with a median of 20 days per year, compared to commercial applicators who applied pesticides an average of 11 years with a median of 45 days per year (Alavanja et al. 1996). Use of personal protective equipment, as well as mixing and application methods, varies by applicator (Karalliedde et al. 2003). Exposure

does not necessarily correlate with dose, and dose is related to a complex set of factors including the route of exposure, chemical properties of the pesticide, its formulation, concentration, and individual metabolism and clearance (Karalliedde et al. 2003). Individual susceptibility to OP toxicity has been associated with genetic characteristics, age, presence of other diseases, smoking, and food intake (Nakajima et al. 2000). However, little is known about gender differences in OP toxicity (Karalliedde et al. 2003), and there have been no studies focused solely on female farm residents, their exposures to farm chemicals, and the potential neuropsychological effects of exposure to pesticides.

The epidemiology of depression in women is different than in men (Kornstein 1997). Women have a lifetime prevalence rate of depression of 20% compared to 10% in men (Bhatia et al. 1999). It has long been observed that depression occurs twice as frequently in women as it does in men. Whether this is due to diagnostic criteria, propensity to seek treatment, biological differences between men and women, or a combination of all of these explanations continues to be debated (Kornstein 1997). Depression in women differs from that in men in that it tends to occur at earlier ages, lasts longer, and is more frequently associated with stressful life events (Aneshensel 1985; Bhatia et al. 1999; Kornstein 1997; Kornstein et al. 1996).

Risk factors for depression in women include a family or personal past history of mood disorders, loss of a parent before age 10, childhood history of physical or sexual abuse, persistent psychosocial stresses and the loss of a social support system (Bhatia et al. 1999; Kornstein 1997). Farm spouses have additional burdens associated with financial hardship, heavy seasonal workloads, having a non-farm job to alleviate financial

concerns, and exposures to chemicals that are known to be associated with depression and anxiety (Carruth et al. 2002; Engberg 1993). They fill-in when labor is required during planting and harvesting, take care of vegetable gardens and animals, tend to the children and manage the household (Carruth et al. 2002; Engberg 1993). Persistent psychosocial stress may result in an elevated risk of depression in farm spouses compared to their non-farm counterpart (Aneshensel 1985).

Few studies have characterized the types of hazards that women encounter on the farm and the nature of their chemical and environmental exposures. In a review of American women and farm work, of the 2,377 women farm residents studied, 5% regularly applied fertilizers, herbicides or insecticides, 12% reported occasionally applying the chemicals and 83% reported never applying any insecticide (Sachs 1987). However, 74% reported regularly taking care of vegetable gardens or animals for family consumption, 14% reported occasionally doing this and 12% reported never doing this (Sachs 1987). In a cross-sectional survey conducted in Colorado between 1993 and 1997, approximately 37% of spouses of principal farm operators reported working in crop production (Stallones 2003). Stallones reported that women worked many hours on the farm but worked fewer hours and did different tasks compared to men on the farms. A cross-sectional survey of 657 farm women in southeast Louisiana reported that 88.9% were involved in the management and oversight of the farm operation, 60.3% cared for farm animals, 70.8% cared for and used farm equipment, and 42.5% were involved in crop management (Carruth et al. 2002).

Previously published data from the AHS showed that 68% of Iowa spouses and 53% of North Carolina spouses mixed or applied pesticides with a median frequency of

50 lifetime days (range 2.5 to 7,000) (Kirrane et al. 2004). Occupational survey data suggests that women on farms do not perceive much of the work they do as farm work (Engberg 1993). In addition to helping in the fields, they are directly exposed to pesticides applied in their homes, on their lawns, and in their gardens. Indirect exposures include laundering the clothing of their spouses, exposure to chemicals carried into the home by those who had been working in the fields, and by-stander exposures due to living in proximity to fields or orchards that are sprayed (Gladen et al. 1998).

Previous studies have focused on male pesticide applicators when examining neurological outcomes and pesticide exposure because so few women work as professional pesticide applicators. The AHS provides the opportunity to study pesticide exposures occurring in the course of farm work undertaken by women and to test whether low-dose pesticide exposure over time or acute pesticide poisonings are associated with diagnosed depression requiring medication in a population of female farm spouses.

METHODS

Study Population

Data for this study are from the AHS, a prospective cohort study, with enrollment of 32,347 spouses of farmer pesticide applicators. The AHS was designed to follow 89,658 applicators and the spouses of farmer applicators for the purpose of studying health outcomes in an agricultural population (Alavanja et al 1996). The study population was from Iowa and North Carolina, and baseline enrollment occurred between 1993 and 1997. The study enrolled 52,395 private farmer applicators and 32,347 spouses of farmer applicators in Iowa and North Carolina, and 4,916 commercial applicators in Iowa. The spouses of farmer pesticide applicators, who are the focus of this paper, responded to

detailed questionnaires and returned them by mail. Of the 82% of eligible private applicators enrolled into AHS, 81% were married and 76% of eligible spouses were enrolled into the spouse study (Kirrane et al. 2004).

This case-control study uses questionnaire data collected from the spouses of farmers who sought a restricted-use pesticide license from Cooperative Extension services or Departments of Agriculture in Iowa and North Carolina (Alavanja et al. 1996). Data were also collected from commercial applicators in Iowa, but data were not collected on spouses of commercial applicators. Cases were defined as female spouses who self-reported being diagnosed with depression requiring medication. Controls were female spouses who stated that they had never been diagnosed with depression requiring medication.

Exclusions from the Study Population

Because depression in women is very different than depression in men, and because so few spouses were males, male spouses were excluded from the analyses. Spouses who had missing responses to the diagnosed depression question were excluded from the study. Those who reported a previous lead or solvent poisoning (Walker 2000) or head injury were excluded from the study due to the strong association with depression. Only individuals 18 years of age or older at the time of enrollment were included in the study.

Study Variables

Diagnosed depression requiring medication was a dichotomous variable coded as yes or no. The question used was "Has a *DOCTOR* ever told you that you had been diagnosed with depression requiring medication?" Following this question was "IF YES,

How old were you when the doctor first told you?" Possible responses to the age at diagnosis question were younger than 20 years, 20-39 years, 40-59 years, and 60 years or older. Demographic characteristics included in the analyses were age, state of residence, education, race, and Hispanic ethnicity. Personal and behavioral variables included cigarette smoking and alcohol use, visiting a physician during the past 12 months and reported medical conditions. No information was provided regarding income or debt. Previous work analyzing the differences between Iowa and North Carolina farm residents identified the types of crops grown and animals raised and the size of the farms to be different between the two states (Alavanja et al. 1996). State of residence was used as an indicator variable to adjust for geographic differences, such as crops grown, farm size and farm practices. Race was coded as white, black, American Indian/Alaskan native, Asian/Pacific Islander and unknown and was used as a dichotomous variable in the models with whites being the reference group. Hispanic ethnicity was included in the models as a separate variable. Education was analyzed as whether or not the respondent had completed high school. Age was categorized into four groups as follows: less than 40 years, 40 to 49 years, 50 to 59 years and greater than 59 years, with those under 40 as the reference group. All respondents were married to farmer applicators. Alcohol use was categorized into never/rarely, monthly, weekly or daily. Smoking status was analyzed as never, past, or current.

Farm and work history information included the number of years lived or worked on a farm and whether the respondent lived on a farm ten years prior to enrollment into the study. Employment history included whether the respondent was employed off the farm and the amount of time spent at that job. The respondents were also asked about

twenty potential occupational exposures from the non-farm job held the longest including pesticides, solvents and heavy metals. Exposure to solvents was included in the multivariable models as a dichotomous variable to adjust for confounding due to the association of solvents and neurological effects (Walker 2000).

Subjects in observational studies of exposure effects are not randomly assigned into groups and they may differ systematically with regard to variables that are related to the exposure and/or the outcome. For example, individuals with a medical condition may be less likely to be exposed to pesticides because they are too ill to perform farm work, or they may choose not to engage in activities that result in chemical exposures. If the individual is depressed due to the medical condition, it may appear that being unexposed is associated with depression when in fact it is the medical condition that is the true cause of the depression. The medical condition may act as a confounder of the true association between pesticide exposure and depression and should be adjusted. A propensity score adjusts for the potential bias attributable to having a medical condition and being diagnosed with depression (Rubin 1997). The propensity score was used to reduce 44 possible medical conditions to a single representative variable predicting the probability of being diagnosed with depression and having one or more medical conditions. The propensity score was calculated for cases and controls using the spouse population after exclusions by saving the predicted probability of the dependent variable for each respondent after running a stepwise regression model predicting the probability of diagnosed depression. The predicted probability is a number between zero and one and represents the relationship between one or more medical conditions and diagnosed depression in each respondent. Medical conditions associated with being diagnosed with

depression at the 5% significance level were retained in the step-wise analysis, and the probability of being diagnosed with depression was output for each spouse.

Since an increased number of visits to a physician may increase the probability of being diagnosed with depression, the number of visits to a doctor was used to adjust for this potential bias. A combined variable was created from the number of doctor visits and being below or above the median of the propensity score to adjust for both the number of visits to a physician and having a medical condition. Models using these two variables were compared to determine whether visits to a doctor was adjusting sufficiently for both a possible diagnosis bias and having a medical condition.

The percent of mixing or applying of pesticides the respondent participated in on the farm was asked as whether they did this less than 50% or more than 50% of the time that pesticides were applied. Data on the use of specific chemicals were obtained as whether they mixed or applied certain herbicides, insecticides, fumigants or fungicides. Years of mixing or applying pesticides was asked using the following six categories: 1 or less; 2 to 5; 6 to 10; 11 to 20; 21 to 30; and more than 30 years. Similarly, the number of days per year of application was asked as seven ordinal categories as follows: less than 5; 5 to 9; 10 to 19; 20 to 39; 40 to 59; 60 to 150; and more than 150 days per year. Exposure to pesticides was measured using lifetime years of mixing or applying pesticides and the number of days per year the applications were done. Categorical values were coded using the means of each interval. For this reason, exposure analysis was limited to the approximate number of days of personally mixing and applying pesticides and whether the study participant reported an acute pesticide poisoning. The pesticide-poisoning question was part of the medical history as: "Has a *DOCTOR* ever

told you that you had been diagnosed with pesticide poisoning?" If the respondent checked yes, he or she was asked, "How old were you when the doctor first told you?" Possible responses were younger than 20 years, 20-39 years, 40-59 years, or 60 years or older. Other exposures examined were having personally treated the home or lawn for pests.

Total lifetime exposure time was calculated by multiplying the number of years of mixing and/or applying and the number of days pesticides were personally mixed and applied and was dichotomized into low-level and intermediate-level exposures in this population. Pesticide poisoning was considered a high-level exposure for purposes of this analysis, resulting in four exposure categories: never mixing or applying pesticides; a low number of exposure days; an intermediate number of exposure days; and a diagnosed pesticide poisoning. The reference group were those who reported never handling pesticides.

Analysis of the data

The propensity score was used to reduce 44 medical conditions, excluding a diagnosed pesticide poisoning, to a single composite variable representing the probability of being diagnosed with depression given the presence of a medical condition. Medians of the distribution of the propensity score were calculated separately for Iowa and North Carolina respondents because there appeared to be state differences in having a medical condition and being diagnosed with depression. The respective medians were used to create a new variable to classify those with low and high probabilities of being depressed and having a medical condition. The dichotomized propensity score was combined with the number of doctor visits in the past year to adjust for having a medical condition and

being depressed, and seeking medical care often enough to be diagnosed with depression by a physician. The first group had a low probability of having a medical condition and being depressed, regardless of the number of visits to a doctor. These were subjects who did not have a serious medical condition associated with diagnosed depression. The second category included those subjects who saw a doctor at least once in the past year and had a propensity score that was above the median, suggesting that they had a medical condition and were more likely to be diagnosed as depressed compared to those without a doctor visit. The third category, the most at-risk for a diagnosis of depression, were those who saw a doctor more than once and had a propensity score that was above the median, indicating a serious medical condition and a high probability of being diagnosed as depressed compared to the other two groups.

Demographic, behavioral and exposure characteristics were compared between cases and controls in univariate analyses using chi-square tests for dichotomous categorical data and logistic regression for polytomous variables. Age, cigarette smoking, alcohol use and visits to a doctor were coded as indicator variables to obtain odds ratios (OR) and 95% confidence intervals (CI).

The Wilcoxon-Mann-Whitney test (WMW), a nonparametric test for differences in the distribution of ordinally-scaled response variables, was used to analyze differences in exposure time in those depressed and not depressed and those with a pesticide poisoning and those without a poisoning. Multivariable logistic regression analysis was used to determine whether an exposure category was associated with diagnosed depression, adjusting for the demographic, behavioral, and exposures, such as solvents, that were significantly associated with depression in the univariate analyses. The chi-

square test statistics and odds ratios, along with 95% confidence intervals, were calculated in both the univariate and multivariate analyses.

An analysis was done separately for Iowa and North Carolina to determine if the results were consistent in each state and to examine any differences that might be present between the two populations. The results for the two states were compared using five different models: (1) unadjusted, (2) age-adjusted, (3) adjusted by age and doctor visits, (4) adjusted by age and combined doctor visits and propensity score, and (5) adjusted by age, combined doctor visits and propensity score and significant variables from the univariate analyses.

The types of pesticide exposures were examined by classifying them into four broad categories: insecticides, herbicides, fumigants and fungicides. Logistic regression analysis was used to obtain the odds ratios and 95% confidence intervals for having mixed or applied a specific type of pesticide and being diagnosed with depression. Further refinement of the pattern of exposure was obtained by using a principal component analysis (PCA) on all fifty pesticides, based on whether a spouse reported using or not using the pesticide. PCA, a data reduction method, was used to group the pesticides most commonly used together by the respondents to facilitate the identification of the most pertinent pesticides. The factors represent individual pesticides that correlated most strongly with the underlying factor and after varimax rotation were completely uncorrelated with one another. Specific chemicals were considered correlated with each factor if they had a factor loading of 40 or greater, which represents approximately a 15% overlap of the variance in pesticide use with the factor. Factors were considered significant if they had eigenvalues greater than one, meaning that they

had greater than a 50% chance of being correlated with the factor. The factors were used in unadjusted logistic regression models to determine whether they were significantly associated with diagnosed depression. A separate PCA analysis was done for each state because of reported differences in pesticide use between Iowa and North Carolina (Alavanja et al. 1999).

All statistical analyses were conducted using SAS, version 8.2, SAS Institute, Cary, North Carolina.

RESULTS

A total of 219 male spouses were excluded from the analyses. Spouses who were missing responses to the diagnosed depression question (n=1608), those who reported a previous head injury (n=1438), solvent poisoning (n=41) or lead poisoning (n=19) were excluded from the study; 218 cases of depression were lost due to these exclusions. After the exclusion of two respondents who were under 18 years of age, 29,074 individuals were included in the study (89.9% of enrolled spouses); there were 2,051 (7.1%) self-reported cases of diagnosed depression requiring medication. More respondents were from Iowa (n=20,073) than from North Carolina (n=9,001). The prevalence of diagnosed depression was greater in North Carolina (8.1%) than in Iowa (6.6 %). Only 63 respondents (0.2%) reported a pesticide poisoning, 44 in Iowa and 19 in North Carolina.

Diagnosed depression was significantly higher in study participants from North Carolina than in those from Iowa ($p<0.0001$) (Table 2.1). The difference was reduced after adjusting for smoking, alcohol use, and number of visits to a doctor. North Carolina women were twice as likely to be current smokers (15.3% vs. 7.9%), were more likely to report never drinking alcohol (72.5% vs. 32.6%), and more likely to visit a physician compared to women in Iowa (54.6% vs. 48.4%) (data not shown). Being non-white

reduced the odds of being diagnosed with depression (Table 2.1). There were very few Hispanic spouses (n=253).

Cases were more likely to be older and past or current cigarette smokers (Table 2.1). Education was not significantly associated with being diagnosed with depression (Table 2.1). Compared to those who drank one to four drinks per week, those who reported never or rarely drinking alcohol had an increased odds of being diagnosed with depression as did those who reported drinking every day or almost every day (Table 2.1). Those who reported working a job off the farm had an increased odds of being diagnosed with depression (Table 2.1), although no difference was observed between cases and controls in the number of years that the study participants worked off the farm (OR 0.99; 95% CI 0.95, 1.03).

This study population was relatively healthy with a median propensity score of four in Iowa and six in North Carolina, indicating that North Carolina had a greater number of study participants who were diagnosed with depression due to a medical condition. As expected, the number of doctor visits significantly predicted being diagnosed with depression with a 2.5-fold increase from one visit per year to more than one visit per year (Table 2.1). The percent of women below and above the median propensity score was reversed in cases and controls, such that 60% of the controls were below the median and 60% of the cases were above the median. Using state-specific distribution medians, those with a propensity score below the median had the lowest probability of being diagnosed with depression, regardless of the number of visits to a doctor. The group in the middle had a propensity score above the median indicating a medical condition and had fewer than two visits to a doctor. A highly significant odds of

being diagnosed with depression was observed in those with a propensity score above the median and two or more visits to a doctor. The results indicated that having a medical condition and being diagnosed with depression was also a function of the number of doctor visits. The greater the number of visits to a doctor the more likely is a diagnosis of depression or, alternatively, a diagnosis of depression results in more visits to a doctor.

Table 2.2 shows the number and percent of reported health conditions and the odds ratios and 95% confidence intervals associated with diagnosed depression in the study population. High blood pressure was the most commonly reported medical condition, followed by pneumonia. Multiple sclerosis and non-Hodgkin's lymphoma showed the strongest association with diagnosed depression. The severity of disease ranged from allergy to cancer. Stratification by state showed very similar disease distribution with no significant differences in the types of medical conditions by state (data not shown).

Table 2.3 describes the farm work done by women in the study. The only difference between cases and controls was a significant trend in the number of days worked during the last growing season, with cases tending to work fewer days than controls (chi-square 21.6, $p<0.0001$). Approximately 50% of the women actively participated in farm work, the majority working 10 to 100 days per growing season. The work most frequently done involved tilling, planting and harvesting crops.

Table 2.4 describes reported farm activities resulting in exposure to pesticides and other chemicals. The mean number of years lived on farms did not differ between cases and controls (mean 31.9 years with a standard deviation of 18 years). Cases significantly differed from controls in that they were more likely to have personally mixed or applied

pesticides. Exposure to solvents and personally applying pesticides to the residence was significantly associated with diagnosed depression. Never having used a livestock or poultry pesticide was negatively associated with diagnosed depression. Pesticide exposure time as measured in total days ranged from none to 7,000 days with the cut-off of the 90th percentile being 225 days of exposure. Due to the skewness of the distribution and the extreme values, exposure time was dichotomized into low (</ =225 days) and intermediate categories (>225 days) based on the 90th percentile. Low and intermediate categories of exposure to pesticides without a poisoning showed a slightly elevated odds ratio for diagnosed depression compared to no personal pesticide use. The association with depression was stronger in those who reported an acute pesticide poisoning (OR 3.97; CI 2.18, 7.21). The distributions of exposure time in those with and without depression were significantly different (WMW chi-square 6.5, p=0.0110), but the difference was stronger for pesticide poisoning and exposure time (WMW chi-square 10.3, p=0.0014). Compared to those who did not report a poisoning, those with a poisoning were significantly more likely to fall into a higher exposure category (OR 1.80, CI 1.26, 2.56). Of the 63 poisonings, 28 reported no exposure time, 25 had exposures of fewer than 226 days and 10 applied greater than 225 days.

Multivariable models are shown in Table 2.5. This model included significant variables from the univariate analyses that remained significant in the multivariable model. Being between 40 and 59 showed an increased risk of depression compared to those older and younger. An elevated odds ratio was observed for state of residence, but the lower limit of the 95% confidence interval contained one. Never drinking alcohol increased the odds of being diagnosed with depression, as did being a past or current

cigarette smoker. Being non-white showed a protective effect on diagnosed depression. Solvent exposure showed a significant association with diagnosed depression, but with a lower odds ratio than those who reported a pesticide poisoning. Both low and intermediate exposure categories without a poisoning showed slightly increased, but non-significant, odds of being diagnosed with depression. Having an acute pesticide poisoning was strongly associated with depression. To examine the association of medical conditions with age on exposure level, a separate analysis excluding those sixty years of age and older was done. After adjusting for the covariates shown in Table 2.5, pesticide poisoning showed a greater association with depression (OR 3.98; CI 1.95, 8.15), as would be expected when the age group with the strongest association between medical conditions and depression is removed from the analysis.

Table 2.6 shows the results of the analysis stratified by state and compares low exposure, intermediate exposure, and pesticide poisoning to those with no personal pesticide exposure using five different models. Model 1, unadjusted, shows a higher odds ratio for depression among women residing in North Carolina compared to Iowa. Models 2, 3, 4 and 5 are age-adjusted. Age slightly reduced the effect of the association between pesticide exposure and depression. Model 3 adjusts for the number of doctor visits resulting in a reduction in the odds ratio for diagnosed poisoning in North Carolina, but not in Iowa. Using the combined doctor visits and propensity score (Model 4) rather than simply the number of doctor visits did not dramatically change the results of the previous models, but the odds ratio went down in Iowa, and increased in North Carolina compared with using the number of doctor visits. The odds ratio was further reduced in both Iowa and North Carolina models using the doctor visits with the propensity score

and adjusting for smoking, alcohol, solvent exposure and being non-white. After adjusting for all these confounders, the association between depression and pesticide poisoning remained higher among North Carolina women compared to Iowa women. The association between depression and diagnosed poisoning remained statistically significant, yet the low and intermediate level exposures did not.

Modeling four broad categories of pesticide exposures showed that insecticides, fumigants and fungicides were significantly associated with diagnosed depression (Table 2.7). The PCA identified four significant factors (Table 2.8). Factor 1 explained most of the variance of pesticide use with an eigenvalue of 13.9 and showed strong correlations with individual herbicides and one OP, but this factor was not significantly associated with diagnosed depression in a univariate logistic regression model (Table 2.9). Factors 2 and 3 were correlated with OPs, carbamates, and the herbicide 2,4-D. Both of these factors were significantly associated with diagnosed depression in univariate logistic regression models, although the association was weak. Factor 4 was correlated with the fumigant methyl bromide and several fungicides. This factor showed an elevated odds ratio for diagnosed depression, but the confidence interval contained one.

Separate PCA analysis by state showed differences in pesticide exposures (2.10 and 2.11). PCA of North Carolina women, who had a higher odds ratio for pesticide poisoning and depression than Iowa women, showed five significant factors compared to three for Iowa. One factor was significantly associated with depression (OR 1.13; 95% CI 1.04, 1.23) (Table 2.12). The factor loadings for this significant factor showed higher values in North Carolina than in Iowa for malathion and diazinon. An examination of crops grown and animals raised in Iowa and North Carolina identified tobacco as the only

significantly different farm product (tobacco is grown only in North Carolina). When using both Iowa and North Carolina populations, and including only women who personally used pesticides, tobacco was significantly associated with depression (OR 1.33, C.I. 1.09, 1.63). Further examination of pesticides used in North Carolina by women on tobacco farms did not isolate any one type of pesticide used on tobacco plants, but a great variety of pesticide classes.

DISCUSSION

Stallones et al. (1995) found a prevalence of depressive symptoms of 11.1% in female farm residents in Colorado based on the Center for Epidemiologic Studies Depression (CES-D) scale. This prevalence rate is lower than the 20% prevalence of depression in the general female population. Scarth et al. (2000) found a higher prevalence of depressive symptoms in male and female Iowa farmers (12.2%) than in Colorado farmers (7.4%). Carruth (2002) found a 24% prevalence of depressive symptoms in southeast Louisiana farm women and Linn and Husaini (1987) found an overall prevalence of 19% in a survey of Tennessee farm residents. The prevalence of depressive symptoms in the farming community may differ by state. The prevalence of diagnosed depression in this study population of 6% to 8% indicates that there may be some underreporting of depression in this study. However, in the Linn and Husaini study only 5.3% of the farmers interviewed indicated they had ever utilized a mental health center due to being depressed. The 6% to 8% prevalence rate for diagnosed depression in this AHS study is comparable to the Linn and Husaini finding. Future research should examine the factors that prompt people in the farming community to seek mental health assistance.

Using diagnosed depression as the outcome may select for those with depressive symptoms of the type described by Aneshensel (1985) as recurrent-chronic rather than an isolated episode. Because symptoms come and go, previous cross-sectional studies using standardized depression scales may be identifying both types of depression without being able to distinguish between them (Aneshensel 1985). A report by May (1993) suggests that agricultural workers underutilize mental health resources due to the complexities of farm life. A female farm spouse who seeks treatment for depression may have a long history of being symptomatic and seeks medical attention because the condition is affecting her ability to function. If this holds for the AHS female spouse population, using diagnosed depression may be a more specific outcome measure in that it selects for those who have a serious mood disorder.

In this study, pesticide poisoning was significantly associated with depression after adjusting for either only the number of visits to a doctor in the past year and combining number of visits with the probability of being diagnosed or depression due to having another medical condition. The number of visits to a doctor in the past year is a crude indicator of health status, but a similar result was found when further adjusting for the probability of having another medical condition or disease in the separate analyses by state. The higher median propensity score indicates a greater number of medical conditions associated with diagnosed depression in North Carolina women compared to Iowa women. North Carolina women had a higher prevalence of diagnosed depression which may result in more frequent physician visits due to medication used to adjust depression. Physicians in North Carolina may have been more likely to diagnose depression in women compared to physicians in Iowa, or it may be that more visits to a

doctor due to another medical condition increased the probability of being diagnosed with depression. Unmeasured risk factors, such as economic conditions, resulting in greater psychosocial stress may have resulted in a higher prevalence of depression in North Carolina women.

Stratifying by state showed similar associations of low and intermediate categories of exposure and diagnosed depression between Iowa and North Carolina women, but this was not true for the poisoning category. North Carolina women had an odds ratio for diagnosed depression in those who reported a pesticide poisoning that was greater than two-fold higher than Iowa women, despite the fact that the percentage of reported poisonings were nearly identical in the two states (Iowa: 0.22%; North Carolina: 0.21%). A previous study from AHS noted that Iowa and North Carolina farmer applicators used dissimilar types of pesticides, applied them differently, and that a higher percentage of Iowa farmer applicators used personal protective equipment (PPE) than in North Carolina (Alavanja et al. 1999). Both the PCA results showing different patterns of pesticide use in Iowa and North Carolina and the association of depression with growing tobacco in those personally mixing or applying pesticides provides evidence for this explanation. Although this study focused on the spouses of Iowa and North Carolina private farmer applicators, the pesticide exposures of the spouses would be similar, but at lower levels, than their husbands, and the spouses probably exercise similar practices in the use of PPE as their husbands do. These differences may explain the finding that North Carolina women had a higher odds of diagnosed depression associated with pesticide poisoning than Iowa farm spouses did if, in fact, poisoning from specific compounds increases the risk of depression. North Carolina spouses might be using

pesticides that are more likely to cause neuropsychological effects and are likely to receive a higher dose due to the application methods and a reduced use of PPE. The finding that low and intermediate categories of exposure showed the same result in both populations strengthens the argument that the pesticide poisoning is the exposure primarily associated with depression.

Several lines of evidence suggest that the association of pesticides with depression may be related to insecticides rather than herbicides. First, never having used an insecticide was negatively associated with diagnosed depression. Second, using insecticides was significantly associated with diagnosed depression. Finally, OPs and carbamates were the most likely agents involved in the association between pesticides and diagnosed depression based on the PCA analysis. Herbicides appeared to be the least likely pesticides associated with depression, but fungicides cannot be ruled out as playing a role. Lastly, the stronger association of acute pesticide poisoning and depression in North Carolina can be explained by a greater use of specific OPs and carbamates. Given these four pieces of evidence, it seems unlikely that chance is the explanation of the observed associations.

Duration of exposure was relatively low in this population. Those who did not have a pesticide poisoning had a median number of exposure days of 50.8 and a mean number of days of 160, a highly skewed distribution. Those who reported a pesticide poisoning had a median number of exposure days of 8.8 and a mean of 237.8 days of exposure. The low exposures in this population make it unlikely that women had developed a tolerance to OPs and were underreporting a pesticide poisoning. The low median number of days in the poisoned groups also makes it unlikely that low-dose,

long-term exposure is acting as a confounder on pesticide poisoning, particularly since 28 of the 63 poisonings were in the no exposure category and another 25 in the low exposure category. At the low and intermediate levels of pesticide exposure observed in this population, there does not appear to be an association with diagnosed depression.

Treating one's home for pests significantly predicted diagnosed depression in this study. Since 18.8% of those who responded that they did this activity also reported never having personally mixed or applied pesticides suggests that exposures may be underreported in these farm residents. In response to the questions "Are pesticides ever used in your vegetable garden?" and "Are pesticides ever used on fruit in your orchard or garden?" 16,739 spouses responded yes. Of the 75.9% who reported pesticide use on their gardens or fruit trees, 6,363 or 38.9% reported never mixing or applying pesticides. Assuming that those who also apply pesticides to their house or lawn are applying to their garden or fruit trees, means that over a thousand women underreported pesticide exposures.

Cases were significantly more likely to have mixed and/or applied pesticides less than 50% of the time that pesticides were used. Applying pesticides less than 50% of the time was significantly associated with working a job off the farm (OR 1.27; CI 1.16, 1.40), although this was not true for mixing pesticides. An increased number of years living on the farm meant a greater likelihood of mixing and applying pesticides and the greater number of years on the farm, the less likely the spouse was to report a pesticide poisoning (OR 0.98; CI 0.97, 0.99). It is possible that working a job off the farm and applying pesticides means that women were at greater risk of a poisoning because they

were in a hurry to get the work done and had less focused time to get the job done. It may also indicate less experience at applying pesticides.

Precise information was not obtained on when the diagnosis of depression was made and therefore whether the pesticide poisoning occurred prior to the diagnosis of depression is not known. Age at diagnosis was asked in 20-year categories, and although no one reported a diagnosed depression prior to a pesticide poisoning using these broad categories, there is no way to determine the time frame between the poisoning event and when the diagnosis of depression was made.

Limitations in this study include the fact that exposures without a pesticide poisoning may have been too low to see a true "chronic" effect of pesticide exposure, however, this comes with the trade-off of having sufficient time to develop a tolerance to OPs, as has been reported in the literature. Having a low exposure time may make recall of a pesticide poisoning more accurate, because the event would be more prominently remembered in someone who does not apply pesticides every day as a routine occupational task. After experiencing an acute poisoning, a farm resident may choose not to mix or apply pesticides again.

Both diagnosed depression and diagnosed pesticide poisoning were self-reported, and no specific details concerning the time and circumstances of the diagnoses were available on the questionnaires. The nature of the depression, whether it was a major depressive episode or recurrent depression, and the pesticide that resulted in the poisoning are unknown. Any measures of risk factors, such as lack of social support or negative life events which may have precipitated the depression, cannot be determined,

and whether these might be differentially distributed between those who did and did not apply pesticides is also unknown.

Despite these limitations, this study suggests that, although women spouses work fewer hours than their husbands mixing and applying pesticides, they are at risk of experiencing neuropsychological effects due to a pesticide poisoning. Depression was more strongly associated with a pesticide poisoning than with exposure without a poisoning, and this study provides preliminary evidence that OPs and carbamates may be responsible for the associations reported between pesticides and depression. These findings are consistent with previously reported results (Daniell et al. 1992; Fielder et al. 1997; Rodnitzky et al. 1975) where exposure to OPs without a poisoning showed very little or no neurological effects, but in the presence of a high level of exposure or acute poisoning, neurological effects were observed (Amr et al. 1997; Reidy et al. 1992; Rosenstock et al. 1991; Savage et al. 1988). Moreover, because women do not perceive themselves as the principal operator of the farm and because they are involved in activities that they may not view as farm work, they may underreport pesticide exposures resulting from spraying their residences and gardens. This study highlights the importance of preventing pesticide poisonings in those occupationally exposed since the chronic effects of those poisonings may contribute to high rates of depression into the future.

Table 2.1. Demographic differences between cases and controls in 29,074 Iowa and North Carolina female spouses of farmer pesticide applicators, AHS, 1993-1997.

Characteristic	Cases (n=2051) n (%)	Controls (n=27023) n (%)	χ^2 (p-value)	OR (95% CI)
State of residence				
Iowa	1322 (64.5)	18751 (69.4)	reference	reference
North Carolina	729 (35.5)	8272 (30.6)	21.7 (<0.0001)	1.25 (1.14, 1.37)
Race				
White	2028 (99.0)	26502 (98.2)	Reference	reference
Non-white	20 (1.0)	477 (1.8)	7.09 (0.0078)	0.55 (0.35, 0.86)
Hispanic ethnicity	16 (0.8)	237 (0.9)	0.20 (0.6572)	0.89 (0.54, 1.48)
Age quartiles				
< 40	485 (23.6)	8500 (31.5)	Reference	reference
40 – 49	666 (32.5)	7672 (28.4)	46.2 (<0.0001)	1.52 (1.35, 1.72)
50 – 59	575 (28.0)	6361 (23.5)	51.9 (<0.0001)	1.58 (1.40, 1.80)
> 59	325 (15.9)	4490 (16.6)	10.3 (0.0013)	1.27 (1.10, 1.47)
High school graduate	1682 (93.8)	23069 (94.9)	3.76 (0.0524)	1.22 (1.00, 1.49)
Worked job off the farm	1853 (91.2)	23785 (89.0)	9.78 (0.0018)	1.29 (1.10, 1.51)
Propensity score*				
≤ median	812 (39.6)	16538 (61.2)	reference	reference
> median	1239 (60.4)	10485 (38.8)	369.9 (<0.0001)	2.41 (2.20, 2.64)
Doctor visits in past year				
None	137 (6.7)	5850 (21.8)	reference	reference
Once	376 (18.4)	7955 (29.6)	48.1 (<0.0001)	2.02 (1.66, 2.46)
More than once	1530 (74.9)	13043 (48.6)	316.6 (<0.0001)	5.01 (4.19, 5.98)
Propensity score and doctor visits*				
Score ≤ median	812 (39.7)	16538 (61.3)	reference	reference
> median/ 0 or 1 visits	238 (11.6)	4006 (14.9)	6.04 (0.0140)	1.21 (1.04, 1.40)
> median/> 1 visit	995 (48.7)	6432 (23.8)	535.0 (<0.0001)	3.14 (2.85, 3.46)
Alcohol Use				
Never/rarely	979 (48.2)	11979 (44.8)	8.90 (0.0029)	1.28 (1.09, 1.51)
< 3 per month	831 (40.9)	11526 (43.1)	2.11 (0.1462)	1.13 (0.96, 1.33)
1 – 4 per week	184 (9.1)	2884 (10.8)	reference	reference
Every /almost every day	36 (1.8)	360 (1.3)	5.57 (0.0183)	1.57 (1.08, 2.28)
Cigarette smoking				
Never	1294 (64.8)	19279 (73.0)	reference	reference
Past	417 (20.9)	4560 (17.3)	27.8 (<0.0001)	1.36 (1.21, 1.53)
Current	286 (14.3)	2572 (9.7)	54.1 (<0.0001)	1.66 (1.45, 1.90)

* Reference group is those with less than or equal to the median propensity score of 4 for Iowa and 6 for North Carolina. The second group consists of those with one or fewer visits to the doctor and a score greater than the median. The third group includes those with more than one visit to the doctor and a score greater than the median.

Table 2.2. Health conditions associated with diagnosed depression in 29,074 Iowa and North Carolina female spouses, AHS, 1993-1997.

Health condition	n (%)	OR (95% CI)
Angina	652 (2.2)	1.35 (1.06, 1.71)
Arrhythmia	1644 (5.7)	1.50 (1.27, 1.77)
Bronchitis	1151 (4.0)	1.69 (1.42, 2.02)
Kidney infection	545 (1.9)	2.27 (1.80, 2.85)
Eczema	1341 (4.6)	1.47 (1.22, 1.76)
Epilepsy	205 (0.7)	1.79 (1.19, 2.69)
Allergy	2760 (9.5)	1.45 (1.27, 1.66)
High blood pressure	4308 (14.8)	1.37 (1.22, 1.54)
Mononucleosis	1542 (5.3)	1.69 (1.43, 2.00)
Multiple sclerosis	97 (0.3)	3.49 (2.12, 5.73)
Pneumonia	3230 (11.1)	1.48 (1.31, 1.68)
Rheumatoid arthritis	1188 (4.1)	1.81 (1.52, 2.16)
Stroke	198 (0.7)	1.78 (1.22, 2.61)
Graves	251 (0.9)	2.10 (1.48, 3.00)
Thyroid	1682 (5.8)	1.50 (1.28, 1.76)
Colitis	314 (1.1)	1.69 (1.21, 2.34)
Non-Hodgkin's lymphoma	52 (0.2)	2.84 (1.39, 5.84)
Cancer	1103 (3.8)	1.26 (1.03, 1.55)

Table 2.3. Percents and numbers of female spouses who performed specific farm tasks and hours spent at the tasks during the previous growing season, and odds ratios and 95% confidence intervals comparing farm work tasks between cases and controls, AHS, 1993-1997.

Task	Total % (n)	Cases % (n)	Controls % (n)	OR (95% CI)
Till the soil	23.8 (6855)	22.1 (448)	24.0 (6407)	0.90 (0.81, 1.00)
Plant	23.9 (6872)	23.7 (479)	23.9 (6393)	0.99 (0.89, 1.10)
Apply fertilizer, manure	11.4 (3276)	11.8 (237)	11.4 (3039)	1.04 (0.90, 1.20)
Apply chemical fertilizer	11.1 (3177)	11.9 (240)	11.0 (2937)	1.09 (0.95, 1.26)
Drive combines/harvester	10.6 (3037)	9.7 (194)	10.7 (2843)	0.90 (0.77, 1.04)
Hand pick crops	25.3 (7259)	26.8 (541)	25.2 (6718)	1.09 (0.98, 1.20)
No. of days of farm work during last growing season*				
None	49.2 (14130)	53.3 (1085)	48.8 (13045)	reference
< 10	19.6 (5626)	19.6 (399)	19.6 (5227)	0.93 (0.82, 1.04)
10 - 30	17.5 (5040)	15.7 (320)	17.7 (4720)	0.82 (0.72, 0.94)
31-100	11.0 (3173)	9.4 (192)	11.2 (2981)	0.78 (0.67, 0.92)
> 100	2.7 (779)	1.9 (39)	2.8 (740)	0.64 (0.46, 0.89)

* Significant by the Mantel-Haenszel statistic for trend (chi-square 21.6, $p<0.0001$)

Table 2.4. Differences in pesticide exposures between cases and controls in 29,074 Iowa and North Carolina female spouses of farmer pesticide applicators, AHS, 1993-1997.

Characteristic	Cases (n=2051) n (%)	Controls (n=27023) n (%)	χ^2 (p-value)	OR (95% CI)
Lived on farm 10 years ago	1631 (79.8)	21796 (80.8)	1.42 (0.2331)	0.93 (0.84, 1.04)
Ever personally mixed or applied pesticides	1197 (58.4)	14974 (55.4)	6.70 (0.0096)	1.13 (1.03, 1.24)
% of time personally mixed pesticides				
Never	1188 (67.2)	16310 (70.1)	Reference	reference
< 50%	368 (20.8)	4231 (18.2)	8.16 (0.0043)	1.19 (1.06, 1.35)
50% or more	212 (12.0)	2714 (11.7)	0.82 (0.3663)	1.07 (0.92, 1.25)
% of time personally applied pesticides				
Never	918 (52.0)	12805 (55.2)	Reference	reference
< 50%	468 (26.6)	5649 (24.3)	6.01 (0.0142)	1.16 (1.03, 1.30)
50% or more	378 (21.4)	4762 (20.5)	2.58 (0.1083)	1.11 (0.98, 1.25)
Exposure levels*				
Never mixed/applied	1162 (56.9)	16134 (60.0)	Reference	reference
Low: 1-225 days	716 (35.0)	8980 (33.4)	4.26 (0.0391)	1.11 (1.01, 1.22)
Intermediate: >225 days	152 (7.4)	1736 (6.4)	4.72 (0.0298)	1.22 (1.02, 1.45)
Diagnosed poisoning	14 (0.7)	49 (0.2)	20.5 (<0.0001)	3.97 (2.18, 7.21)
Exposed to solvents	227 (11.1)	2160 (8.0)	23.9 (<0.0001)	1.43 (1.24, 1.66)
Never used livestock or poultry pesticides	1819 (88.7)	24643 (91.2)	14.8 (0.0001)	0.76 (0.66, 0.87)
Never used herbicides	1212 (59.1)	16568 (61.3)	4.00 (0.0458)	0.91 (0.83, 1.00)
Treated home for pests	658 (32.1)	7490 (27.7)	18.0 (<0.0001)	1.23 (1.12, 1.36)
Treated lawn for pests	249 (12.1)	2893 (10.7)	4.07 (0.0436)	1.15 (1.00, 1.32)

* The exposure levels are: reference group never personally applied or mixed pesticides; low exposure includes those exposed to 225 days or less (the 90th percentile); the intermediate group is those with > 225 days of exposure; diagnosed poisoning group reported a pesticide poisoning. Never mixed/applied, low and intermediate exposure was significant in a test for trend (chi-square 6.86, p=0.0088)

Table 2.5. Multivariable logistic regression analysis of exposure levels and diagnosed depression adjusting for demographic and behavioral characteristics shown to be significant in the univariate analysis in 1970 cases and 25959 controls, AHS, 1993-1997.

Variable	Chi-square (p-value)	OR (95% CI)
Age		
< 40	reference	reference
40 – 49	43.9 (<0.0001)	1.53 (1.35, 1.73)
50 – 59	36.1 (<0.0001)	1.50 (1.31, 1.71)
> 59	1.60 (0.2062)	1.11 (0.95, 1.29)
State of residence	2.57 (0.1090)	1.09 (0.98, 1.22)
Doctor visits		
No visits	reference	reference
1 visit	44.8 (<0.0001)	1.99 (1.63, 2.44)
2+ visits	301.6 (<0.0001)	4.97 (4.15, 5.96)
Never/rarely drink alcohol	5.82 (0.0158)	1.14 (1.03, 1.26)
Smoking		
Never smoker	reference	reference
Past smoker	19.7 (<0.0001)	1.31 (1.16, 1.47)
Current smoker	48.7 (<0.0001)	1.66 (1.44, 1.92)
Non-white race	10.3 (0.0013)	0.46 (0.29, 0.74)
Exposed to solvents	18.2 (<0.0001)	1.39 (1.20, 1.62)
Lifetime exposure levels*		
Never mixed/applied	reference	reference
Low: $\leq$ 225 days	1.38 (0.2408)	1.06 (0.96, 1.18)
Intermediate: 226-7000 days	0.85 (0.3569)	1.09 (0.91, 1.31)
Diagnosed poisoning	13.2 (0.0003)	3.28 (1.73, 6.22)

* The exposure levels are: reference group never personally applied or mixed pesticides; low exposure includes those exposed to 225 days or less (the 90th percentile); the intermediate group has > 225 days of exposure; diagnosed poisoning group reported a pesticide poisoning.

Table 2.6. Odds ratios and 95% confidence intervals from unadjusted and adjusted logistic regression analysis of being diagnosed with depression and exposure to pesticides by state of residence, female spouses of farmer applicators, AHS, 1993-1997.

Exposure model *	Iowa (n=20073) OR (95% CI)	North Carolina (n= 9001) OR (95% CI)
Model 1: Exposure levels		
Never mixed/applied	reference	reference
Low: </= 225 days	1.08 (0.96, 1.22)	1.28 (1.08, 1.53)
Intermediate: 226-7000 days	1.24 (0.99, 1.54)	1.19 (0.89, 1.59)
Diagnosed poisoning	2.82 (1.25, 6.33)	7.22 (2.83, 18.4)
Model 2: Exposure levels		
Never mixed/applied	reference	reference
Low: </= 225 days	1.06 (0.94, 1.19)	1.27 (1.06, 1.51)
Intermediate: 226-7000 days	1.17 (0.93, 1.46)	1.12 (0.84, 1.50)
Diagnosed poisoning	2.72 (1.21, 6.14)	7.00 (2.73, 17.9)
Model 3: Exposure levels		
Never mixed/applied	reference	reference
Low: </= 225 days	1.03 (0.91, 1.16)	1.21 (1.01, 1.44)
Intermediate: 226-7000 days	1.15 (0.92, 1.45)	1.07 (0.80, 1.44)
Diagnosed poisoning	2.81 (1.22, 6.43)	5.72 (2.19, 15.0)
Model 4: Exposure levels		
Never mixed/applied	reference	reference
Low: </= 225 days	1.04 (0.93, 1.18)	1.22 (1.03, 1.46)
Intermediate: 226-7000 days	1.14 (0.91, 1.43)	1.04 (0.77, 1.40)
Diagnosed poisoning	2.62 (1.15, 5.98)	6.00 (2.25, 16.0)
Model 5: Exposure levels		
Never mixed/applied	reference	reference
Low: </=225 days	1.05 (0.93, 1.18)	1.19 (0.99, 1.42)
Intermediate: 226-7000 days	1.11 (0.88, 1.40)	1.02 (0.75, 1.38)
Diagnosed poisoning	2.43 (1.05, 5.62)	5.25 (1.81, 15.2)

* Model 1, unadjusted; Model 2, age-adjusted; Model 3, adjusted by age and visits to a doctor; Model 4 age-adjusted and combined doctor visits and propensity score variable; Model 5, is model 4 using doctor visits and propensity score and significant variables in Table 2.5.

Table 2.7. Exposure to pesticides based upon ever having used a pesticide in each of the four classes of pesticides in cases and controls and odds ratios for exposure and being diagnosed with depression, AHS, 1993-1997.

Class of pesticide	Cases % (n)	Controls % (n)	OR (95% CI)
Insecticides			
No	58.4 (1198)	62.1 (16780)	
Yes	41.6 (853)	37.9 (10243)	1.17 (1.07, 1.28)
Herbicides			
No	61.8 (1267)	63.7 (17222)	
Yes	38.2 (784)	36.3 (9801)	1.09 (0.99, 1.19)
Fumigants			
No	97.8 (2005)	98.4 (26579)	
Yes	2.2 (46)	1.6 (444)	1.38 (1.01, 1.87)
Fungicides			
No	94.5 (1938)	95.6 (25821)	
Yes	5.5 (113)	4.4 (1202)	1.25 (1.03, 1.53)

Table 2.8. Results of varimax rotated factor analysis of specific pesticide exposures correlated to four significant factors and their factor loadings with the four factors in Iowa and North Carolina women in the AHS, 1993-1997. *

Factor 1		Factor 2		Factor 3		Factor 4	
Herbicide:		**OPs:**		**OPs:**		**Fumigants:**	
atrazine	66	terbufos	52	malathion	63	methyl bromide	50
dicamba	66	fonofos	53	diazinon	47	**Funigicides:**	
cyanazine	68	chlorpyrifos	41	**Carbamate:**		benomyl	41
metalachlor	72	phorate	46	carbaryl	57	chlorothalonil	42
EPTC	58	**Carbamate:**		**Herbicides:**		maneb/mancozeb	50
alachlor	67	carbofuran	54	glyphosate	52	metalaxyl	60
metribuzen	64			2,4-D	47		
petroleum	47						
pendimethalin	62						
imazethapyr	72						
butylate	49						
trifluralin	64						
chlorimum	55						
OP:							
terbufos	45						

*Factors with an eigenvalue of at least 1.0 were retained in the factor analysis. Pesticides with a factor loading of 40 or greater (at least a 15% overlap of the variance in pesticide use with the factor considered) are reported here.

Table 2.9. Results of univariate logistic regression models of the four factor analysis of pesticides used by female spouses in Iowa and North Carolina and the probability of diagnosed depression due to the factor, AHS, 1993-1997.

Factors	Eigenvalue of factor	Squared canonical correlations	Chi-square (p-value)	OR (95% CI)
Factor 1	13.9	0.94	1.10 (0.30)	0.97 (0.93, 1.02)
Factor 2	2.84	0.76	5.51 (0.019)	1.06 (1.01, 1.12)
Factor 3	1.38	0.61	7.28 (0.007)	1.08 (1.02, 1.13)
Factor 4	1.25	0.58	3.65 (0.056)	1.05 (1.00, 1.10)

Table 2.10. Results of varimax rotated factor analysis of specific pesticide exposures correlated to four significant factors and their factor loadings with the four factors in North Carolina women in the AHS, 1993-1997.*

Factor 1		Factor 2		Factor 3		Factor 4	
Herbicide:		**Fumigants:**		**OPs:**		**Pyrethroid:**	
atrazine	61	methyl bromide	40	malathion	67	permethrin	64
cyanazine	60	**Fungicides:**		diazinon	58	**OPs:**	
metalachlor	60	benomyl	48	**Carbamate:**		coumaphos	46
EPTC	42	chlorothalonil	54	carbaryl	71	dichlorvos	56
alachlor	66	captan	44	**Herbicide:**			
metribuzen	63	maneb	52	glysophate	52		
paraquat	41	metalaxyl	48				
pendimethalin	48						
imazethapyr	44						
butylate	51						
trifluralin	50						
chlorimuron	62						
Carbamate:							
carbofuran	40						

*Factors with an eigenvalue of at least 1.0 were retained in the factor analysis. Pesticides with a factor loading of 40 or greater (at least a 15% overlap of the variance in pesticide use with the factor considered) are reported here. A fifth factor was significant but not shown here because only cyanazine with a factor loading of 41 significantly correlated with it.

Table 2.11. Results of varimax rotated factor analysis of specific pesticide exposures correlated to four significant factors and their factor loadings with the four factors in Iowa women in the AHS, 1993-1997.*

Factor 1		Factor 1		Factor 2		Factor 3	
Herbicide:		**OPs:**		**OPs:**		**OPs:**	
atrazine	69	terbufos	51	terbufos	48	malathion	61
dicamba	68	fonofos	43	fonofos	50	diazinon	55
cyanazine	70	chlorpyrifos	49	chlorpyrifos	43	**Carbamate:**	
metalachlor	73	**Carbamate:**		phorate	43	carbaryl	55
EPTC	59	carbofuran	43	**Carbamate:**		**Herbicide:**	
alachlor	70			carbofuran	55	2,4-D	48
metribuzen	64						
petroleum	51						
pendimethalin	66						
imazethapyr	73						
butylate	50						
trifluralin	65						
chlorimuron	56						

*Factors with an eigenvalue of at least 1.0 were retained in the factor analysis. Pesticides with a factor loading of 40 or greater (at least a 15% overlap of the variance in pesticide use with the factor considered) are reported here.

Table 2.12. Factor eigenvalues and results of univariate logistic regression models of three factors in Iowa and four factors in North Carolina from PCA of pesticides used by women in Iowa and North Carolina and the probability of diagnosed depression due to the factor, AHS, 1993-1997.

Factors	Eigenvalue North Carolina	Eigenvalue Iowa	North Carolina OR (95% CI)	Iowa OR (95% CI)
Factor 1	9.79	15.5	0.99 (0.91, 1.08)	1.00 (0.95, 1.06)
Factor 2	3.70	2.44	1.05 (0.97, 1.15)	1.06 (0.99, 1.12)
Factor 3	1.56	1.62	1.13 (1.04, 1.23)	1.06 (1.00, 1.13)
Factor 4	1.36	N/A	1.03 (0.95, 1.13)	N/A

CHAPTER THREE: DIAGNOSED DEPRESSION AND LOW-DOSE, LONG-TERM PESTICIDE EXPOSURES AND VERY HIGH PESTICIDE EXPOSURES IN IOWA AND NORTH CAROLINA FARMER PESTICIDE APPLICATORS ENROLLED IN THE AGRICULTURAL HEALTH STUDY

BACKGROUND

Nerve agents, a diverse group of organic esters of phosphoric acid derivatives first introduced during World War II, inhibit acetylcholinesterase, resulting in a multitude of muscarinic, nicotinic and central nervous system effects. These effects were described in a series of papers published in 1950 (Grob 1950; Wood 1950). As early as 1953, anxiety, withdrawal, and depression were noted after nerve gas poisoning (Grob et al. 1953), but psychiatric sequelae were not fully characterized until 1961 when Gershon and Shaw reported specific symptoms associated with organophosphorus (OP) poisoning (Gershon et al. 1961). Most acute poisonings are associated with OP or carbamate compounds and result in characteristic symptoms resulting from acetylcholinesterase inhibition, including headache, nausea, dizziness, fasciculations, vomiting, abdominal cramps, diarrhea, sweating and lacrimation (Marrs 1993). A few studies have shown long-term neurological effects in the absence of an acute pesticide poisoning (Amr et al. 1997; Farahat et al. 2003; Salvi et al. 2003; Stephens et al. 1995) while some show little or no neurological effects (Albers et al. 2004; Daniell et al. 1992; Fielder et al. 1997). After a number of studies over many years, the nature of the exposure resulting in neuropsychological effects of extended duration remains unclear (Brown et al. 1998; Ray et al. 2001).

Investigators over five decades have consistently reported neuropsychological impairments resulting from pesticide poisonings or high level exposures and, much less consistently, low-level pesticides exposures and possibly intermediate-level, long-term

exposures (Brown et al. 1998). High-level exposure is that which results in measurable acetylcholinesterase inhibition and causes symptoms of OP poisoning (Brown et al. 1998). Low-level exposures do not cause detectable inhibition of acetylcholinesterase activity (Brown et al. 1998; Ray et al. 2001). Carbamates, although inhibitors of acetylcholinesterase, cause less severe poisoning because they are hydrolyzed more rapidly than OPs (Kwong 2002). A number of studies have documented neurological sequelae among farmers, but recently strong associations between pesticide exposure and depression have been identified in those with high-level occupational exposure to pesticides and pesticide poisoning (Amr et al. 1997; Jamal et al. 2002; Stallones and Beseler 2002). Anxiety and depression have been observed in the course of conducting extensive neurological examinations, but have rarely been specifically studied in hypothesis-driven research (Davies 1990).

Levin et al. (1976) studied 13 commercial pesticide applicators and 11 farmers matched to 24 unexposed controls on age and education. The controls in this study were farmers who were evaluated prior to the spraying season and farmers who did not apply pesticides. The investigators found an association between low-level OP exposure and anxiety but not depression in commercial applicators, but neither in farmers (Levin et al. 1976). Anxiety, restlessness and sleep disturbances were more frequent in commercial applicators than in exposed farmers or unexposed farmers. Anxiety and depression scores did not differ between exposed farmers and unexposed farmers (Levin et al. 1976). This result could be due to differences in patterns of exposure in commercial applicators compared to farmer applicators. Farmers spend more years applying, but fewer days per year compared to commercial applicators, who spend fewer years applying and a greater

number of days per year (Alavanja et al. 1996; Arbuckle et al. 2002). Although within a clinically acceptable range, greater plasma cholinesterase inhibition was observed in commercial applicators than in farm applicators (Levin et al. 1976), indicating higher exposures than farmer applicators or less tolerance since they may have been exposed more recently. Misclassification may have occurred if residual effects of OP exposure existed in the control population, some of whom had a recent history of pesticide use. Chronic acetylcholinesterase inhibition in the commercial applicators, without a poisoning, may have resulted in anxiety.

Ames et al. (1995) studied 45 California pesticide applicators who were not permitted to apply pesticides due to having acetylcholinesterase activities that were 70% of their baseline value but were not pesticide-poisoned. Comparing them to 90 friend controls, no significant differences were observed on mood scales measuring tension, depression or anger.

Most studies where subjects have experienced an acute OP poisoning have consistently shown mood disorders for months or even years after the poisoning. In a study of 100 acutely OP-poisoned individuals matched to 100 non-poisoned controls, six of the individuals with a history of OP poisoning were classified as depressed while none of the controls were classified as depressed ($p=0.003$) (Savage et al. 1988). Relatives of cases reported significantly greater depression, irritability, social withdrawal and confusion than did relatives of controls. The 100 poisoned individuals had a mean of nine years since the primary poisoning and 20 reported more than one poisoning event subsequent to the primary event. The strength of this study was the verified inclusion criteria including a documented history of at least one OP poisoning event with

symptoms indicative of OP poisoning confirmed by a physician. Those with an OP poisoning three months prior to the study were excluded, as well as those with conditions associated with neurological impairment, such as alcohol and drug use, having a learning disability and advanced age. This investigation, using an adequate sample size and matched design, of chronic neurological sequelae in those with a past pesticide poisoning showed that both cognitive functioning and mood disorders were present years after an acute poisoning.

Steenland et al. (1994) evaluated 128 individuals with suspected and confirmed OP pesticide poisoning in California. These individuals were evaluated an average of five years post-poisoning and differed significantly from 90 nonpoisoned controls on tests of sustained visual attention and two mood tests measuring tension, depression, anxiety, fatigue, and confusion (Steenland et al. 1994). An analysis of the primary OP pesticide associated with the poisoning and other OP exposures at the time of the poisoning identified associations with phosalone, chlorpyrifos, diazinon, dimethoate, mevinphos and demeton methyl (Steenland et al. 1994).

In a 1995 study of 81 orchard pesticide applicators in New York, who had an average of 20 years of occupational exposure, odds ratios for sleep problems, weight gain or loss, tension or nervousness, depression and nightmares were elevated in exposed applicators compared to population-based controls (Stokes et al. 1995). Although the 95% confidence intervals included one for these symptoms, probably due to imprecision in small sample sizes, many of the symptoms are indicative of depression and anxiety. The primary OP used was guthion, and its metabolite, dimethylthiophosphate (DMTP), was measured to verify exposure (Stokes et al. 1995). Most of the exposed subjects

reported experiencing the classic symptoms of OP poisoning, but history of acute pesticide poisoning was not obtained (Stokes et al. 1995).

A study of Hispanic migrant farm workers included 21 workers who had experienced two documented acute poisonings and were evaluated two years post-poisoning using a neuropsychological battery, medical history questionnaire, and an anxiety and depression scale (Reidy et al. 1992). Compared to 11 matched nonpoisoned controls, the poisoned group experienced significantly more anxiety, depression, and physical symptoms than the controls (Reidy et al. 1992). The pesticide poisonings in the exposed group were due to phosdrin, a mevinphos compound; lannate, a non-OP crop insecticide, and the fungicide maneb (Reidy et al. 1992).

Neurological symptoms in 57 exposed male fruit tree farmers without a history of an acute poisoning were compared to 42 age-matched male controls who were either cranberry/blueberry growers or hardware store owners in New Jersey (Fielder et al. 1997). Those in the highest exposure group had a slower reaction time in their dominant hand, but none of the psychiatric or mood symptoms were different between pesticide exposed and nonexposed controls (Fielder et al. 1997).

Psychiatric disorders were assessed in an Egyptian population of 208 pesticide formulators, 172 pesticide applicators and 223 control subjects matched on location of residence, age, socioeconomic level and education (Amr et al. 1997). Pesticide formulations were made locally, safety measures were poor, and workers were inadequately trained in the safe handling of the chemicals they mixed and applied (Amr et al. 1997). Pesticide exposures included carbamates, synthetic pyrethroids, organophosphates and organochlorines. The randomly selected formulators were

exposed at least forty hours per week, at least nine months per year, for a minimum of two consecutive years. The General Health Questionnaire (GHQ) was used to measure somatic symptoms, anxiety/insomnia, social dysfunction and severe depression, and the DSM-III-R was used for diagnosis and classification of psychiatric morbidity. Compared to controls, the pesticide formulators had significantly more total psychiatric disorders and specifically more cases of depressive neurosis and situational/reactive depression. Symptoms such as irritability were more common in the pesticide formulators than in the controls. Total psychiatric disorders were significantly elevated in pesticide applicators compared to their matched controls.

A cross-sectional study of 761 Colorado farm residents who had experienced an acute pesticide illness severe enough to experience symptoms had an OR of 5.95 (95% CI 2.56,13.84) for having high depressive symptoms after adjusting for gender, health, age, income reduction, education, social support and alcohol (Stallones and Beseler 2002). In this farming population, 3.9% of the male farmers were classified as depressed based on the Center for Epidemiological Studies Depression (CES-D) scale, and there were 69 pesticide poisonings. Depression was significantly associated with headache and/or dizziness, eye and skin irritation, nausea and/or vomiting, chest discomfort and difficulty breathing, suggesting that the association of pesticide poisoning and depression was due to poisoning by OP compounds.

A study of sheep dippers from two areas of the United Kingdom found that severity of neuropathies was associated with increases in anxiety and depression as measured by a battery of psychometric tests, neurological symptoms and quantitative sensory testing (Jamal et al. 2002). An estimate of cumulative exposure to OPs was

calculated based on application methods and years of use. Mood was assessed using the GHQ and the Hospital Anxiety and Depression Scale (HADS). After adjusting for age and verbal IQ, the probable or definite neuropathy group showed evidence of greater anxiety and depression, but no correlation was observed with cumulative measures of OP exposure. In an earlier cross-sectional study of sheep dippers, Stephens et al. (1995) found a greater vulnerability to psychiatric disorders in 146 sheep dippers compared to 143 quarry workers. Sheep dipping may result in higher exposures because the OP solution splashes off the animals during and after immersion and protective clothing is not usually worn (Stephens et al. 1995).

Depression is multifaceted and includes not only dysphoric mood, but also cognitive, perceptual, behavioral, psychomotor and physiological manifestations (Aneshensel 1985). Symptoms reflect these changes and represent the severity of the disorder and even the type of disorder (Aneshensel 1985), making it not surprising that previous studies of neurological effects and pesticides reveal all these aspects. Psychomotor changes are observed when studying reaction times, cognitive changes are observed in attention deficits, physiological manifestations are seen in the reported symptoms related to sleeping disorders and weight gain or loss. Aneshensel found two types of depression in a study conducted in Los Angeles; isolated episodes that are an aberration in normal mood, and recurrent-chronic episodes that show a strong effect on a person's emotional state. Whether this model of depression holds true for the farming community is unknown.

The purpose of this study is to describe pesticide exposure and depression in a large cohort of farm applicators enrolled into the Agricultural Health Study (AHS) from

1993 through 1997 upon applying for a restricted-use pesticide application license in Iowa and North Carolina. A total of 82% of all eligible private applicators were enrolled into AHS resulting in 52,395 farmer applicators (Gladen et al. 1998). An enrollment questionnaire was filled out at the time of recruitment and an additional take-home questionnaire was returned by fewer than half of those who were enrolled (Tarone et al. 1997).

METHODS

Study Population

Data for this study came from the Agricultural Health Study (AHS) which enrolled individuals who sought a license to apply pesticides in Iowa and North Carolina from 1993 through 1997. AHS was designed to follow 89,658 individuals (4,916 commercial applicators, 52,395 farmer applicators and 32,347 spouses of farmer applicators) for the purpose of studying health outcomes in an agricultural population. Although farmer applicators and their spouses were enrolled in both Iowa and North Carolina, commercial applicators were enrolled only in Iowa. Data collected by two questionnaires included exposure to 186 chemicals (50 on the enrollment questionnaire and another 136 on the take-home questionnaire), detailed questions about mixing and application methods, personal protective equipment used, and work and hygiene practices. Data were also collected on demographic characteristics, including race, education, marital status, involvement in farm work, medical conditions, alcohol use and smoking habits.

Figure 7 (see Results in this chapter) contains a description of the study population used in this report. Of the 52,395 farmer applicators answering the enrollment

questionnaire, only 22,916 (44%) returned the take-home questionnaire containing the question asking whether they had ever been diagnosed with depression requiring medication or shock therapy. The distribution of demographic and exposure variables were compared between those who did and did not return the take-home questionnaire to evaluate this potential bias. Additionally, the enrollment question asking how often the study participant felt nervous or depressed and thought it may be related to pesticides was used as an indicator of whether opinions on depression and pesticide use differed between the responders and non-responders. The question asking how often the study participant had visited the doctor as a result of using pesticides was used to gauge whether the reporting of a pesticide poisoning systematically differed between respondents and non-respondents.

Exclusions from the Study Population

Farmer pesticide applicators who were missing responses to the diagnosed depression question and those who reported a previous lead or solvent poisoning were excluded from the study. Only male individuals 18 years of age or older at the time of enrollment were included in the study. Commercial pesticide applicators were excluded from the study because there were only 4,916 enrolled in the study and fewer than half returned the take-home questionnaire. Further, the literature demonstrates that commercial applicators have different patterns of exposure than farmer applicators (Alavanja et al. 1996; Arbuckle et al. 2002). In addition, the commercial applicator questionnaire differed from the farmer applicator questionnaire.

Study Variables

Cases were defined as farmer applicators who self-reported being diagnosed with depression requiring medication or shock therapy on the take-home questionnaire. Controls were farmer applicators who stated that they had never been diagnosed with depression requiring medication or shock therapy and who reported less than one depressive episode a week during the twelve months prior to enrollment into the study. Diagnosed depression requiring medication or shock therapy, the outcome variable, was a dichotomous variable coded as yes or no. The question asked was "Has a *DOCTOR* ever told you that you had (been diagnosed with) depression requiring medication or shock therapy?" Followed by, "IF YES, How old were you when the doctor first told you?" Possible responses to age at diagnosis were younger than 20 years, 20-39 years, 40-59 years and 60 years or older.

The questionnaire asked, "How often, if ever, have you had the following symptoms that you think may be related to your using pesticides?" Possible symptoms were as follows: been excessively tired, had headaches/dizziness, had nausea or vomiting, had skin irritation, had eye irritation, had chest discomfort, and felt nervous or depressed. Possible responses were never/rarely, sometimes and frequently/almost always. The depressive symptom was used to determine if respondents were more or less likely to associate pesticides with depression than non-respondents. The question "As a result of using pesticides, how often have you seen a doctor?" was used to determine whether respondents were more or less likely to have had an unusually high exposure or a pesticide poisoning than non-respondents. Additionally, the pesticide symptoms were

used as an indicator of OP or carbamate sensitization resulting from an unusually high exposure that was not reported as such. Due to small numbers when stratifying by exposure, the response categories were collapsed into never or rarely experienced symptoms and sometimes, frequently or almost always experienced symptoms. The low and intermediate levels of exposure were combined into one category representing those lower than the 90^{th} percentile of exposure time. The symptom was combined with a specific exposure group to create an indicator variable. Those not experiencing the symptom comprised the reference group. Each of the six symptoms was analyzed separately. Five groups were created as follows: (1) never or rarely experienced a symptom; (2) symptom and up to 752 days of exposure; (3) symptom and greater than 752 days of exposure; (4) symptom and an unusually high exposure and (5) symptom and a poisoning.

Demographic characteristics included in the analyses were state of residence, education, race, Hispanic ethnicity and marital status. Personal and behavioral variables included sex, age, smoking behavior, alcohol use, and frequency of visits to a doctor and medical conditions of respondents. Descriptions of the farms where the pesticide applicators resided included the number of acres planted, farm products, involvement in farm work, number of years respondents worked as farmers and the number who worked off the farm. The farm characteristic variables were categorized on the questionnaire and were used in their original form. No information was provided on the questionnaires regarding income, debt or social support, but it is known that Iowa farms are larger than North Carolina farms, North Carolina farms are more diverse than Iowa farms, and different pesticides are used in Iowa and North Carolina (Alavanja et al. 1999). Race was

coded as white, black, American Indian/Alaskan native, Asian/Pacific Islander and unknown and was used as a dichotomous variable in the models with whites being the reference group and nonwhites the at-risk group for diagnosed depression. Hispanic ethnicity was included in the models as a separate variable. Education was analyzed as whether or not the respondent had completed high school. Marital status was coded as married or unmarried including widowed, divorced, separated, or never married. Alcohol use was categorized into never/rarely, one to three times per month, one to four times per week, or almost every day/daily. Smoking status was analyzed as never, past, or current. The number of visits to a doctor because of a health concern was a categorical variable with none, once and more than once as the response categories.

Farm and work history information included the number of years lived or worked on a farm as a categorical variable. Employment history included whether the respondent was employed off the farm and the amount of time spent at the job. The respondents were also asked about twenty potential occupational exposures from the non-farm job held the longest including pesticides, solvents and heavy metals. Exposure to solvents was included in the multivariable models as a dichotomous variable to adjust for confounding due to the association of solvents and neurological conditions (Walker 2000). Personal protective equipment use was included in the univariate analysis of exposure to determine whether cases and controls differed on this work characteristic related to exposure.

Subjects in observational studies of exposure effects are not randomly assigned into groups and they may differ systematically with regard to variables that are related to the exposure and/or the outcome. In this study, those who were diagnosed as depressed may be those who sought medical attention for other reasons, unrelated to depression.

An individual might be depressed due to a medical condition, but it may take several visits to a doctor to recognize that the individual is depressed. A propensity score controls for the potential bias attributable to seeking treatment for a medical condition and being diagnosed with depression because treatment was sought for another condition (Rubin 1997). A propensity score was calculated by saving the predicted probability of diagnosed depression for each respondent calculated from a stepwise regression model. The predicted probability is a number between 0 and 1 and represents the relationship between the multiple medical conditions reported and diagnosed depression. Medical conditions associated with being diagnosed with depression at the 95% confidence level were retained in the step-wise analysis and the probability of being diagnosed with depression was output for each applicator.

Because individuals who visit a doctor for medical treatment more frequently are more likely to be diagnosed with depression than those who do not visit a doctor frequently, the number of doctor visits was used to adjust for this. A variable combining the propensity score and the number of visits to a doctor was used to adjust for both having a medical condition and diagnosis for depression due to the medical condition.

Lifetime exposure time was calculated as the number of days per year the applicator mixed or applied any pesticide multiplied by the total number of years of pesticide use from the enrollment questionnaire. The number of years personally mixed or applied pesticides was asked in the following categories: 1 year or less, 2-5 years, 6-10 years, 11-20 years, 21-30 years, and more than 30 years. Similarly, the days per year of mixing or applying was in categories of: less than 5 days, 5-9 days, 10-19 days, 20-39 days, 40-59 days, 60-150 days and more than 150 days. The variables were coded using

the mean of each category interval. Five exposure categories were created. The reference group included those who never or rarely mixed or applied pesticides (the lowest quartile of the exposure time variable). The lowest quartile to the 90th percentile of exposure time comprised the intermediate exposure level. The 90th percentile and above were considered the highest exposure group, excluding those with an unusually high exposure or acute poisoning. The very high exposure groups were those who had an unusually high exposure and those who had been diagnosed with a pesticide poisoning.

Analysis of the data

Differences between those who did and did not return the take-home questionnaire were compared using the chi-square test for categorical data. Variables used to compare the respondents with the non-respondents included state of residence, being Caucasian, Hispanic ethnicity, age, education, marital status, alcohol and tobacco use, feeling nervous and depressed and thinking that it was related to using pesticides and visiting a doctor as a result of using pesticides. Differences in pesticide exposures were evaluated by the percent of time the mixing and applying of pesticides was personally performed, and the number of days per year and the number of years the farm resident personally applied the pesticide. Differences were considered to be present at the 5% level of significance.

Demographic, behavioral and exposure characteristics were compared between cases and controls in univariate analyses using chi-square tests for categorical data and logistic regression using indicator variables for polytomous categorized variables. Continuous variables that were not normally distributed such as age, propensity score and exposure levels were categorized. The categorical variables age, smoking, alcohol use,

and exposure were coded as indicator variables to obtain odds ratios and confidence intervals for effects over categories. Farm attributes were compared between cases and controls using odds ratios and 95% confidence intervals. Multivariable logistic regression analysis was used to determine whether exposure level was associated with diagnosed depression, adjusting for the demographic, behavioral, and farm characteristics that were significantly associated with depression in the univariate analysis. Odds ratios with 95% confidence intervals were calculated in the multivariate analyses. One analysis used only doctor visits and another used the combined doctor visits and propensity score to adjust for medical conditions and depression.

Stratified analyses were used to examine depression and exposure levels by number of doctor visits, adjusting for significant covariates. Chi-square, p-values, odds ratios and 95% confidence intervals from multivariable stepwise logistic regression models were used to determine the significance of pesticide exposure and depression in different categories of doctor visits. Stratified analyses were also done by state of residence to determine whether the previous findings held in each state and to identify any differences between the states. Odds ratios and 95% confidence intervals were reported for pesticide exposure levels in six difference models.

Separate univariate and multivariable logistic regression models for each reported symptom were used to examine whether depression was associated with symptoms, stratified by exposure level. Significance was tested using odds ratios and 95% confidence intervals. A multivariable analysis, excluding those with two or more symptoms, was run to determine what effect removing those with symptoms would have

on the model. Chi-square, p-values, odds ratios and 95% confidence levels were reported.

All analyses were done using SAS version 8.0, SAS Institute, Cary, North Carolina. All confidence intervals are reported at the 95% level of confidence.

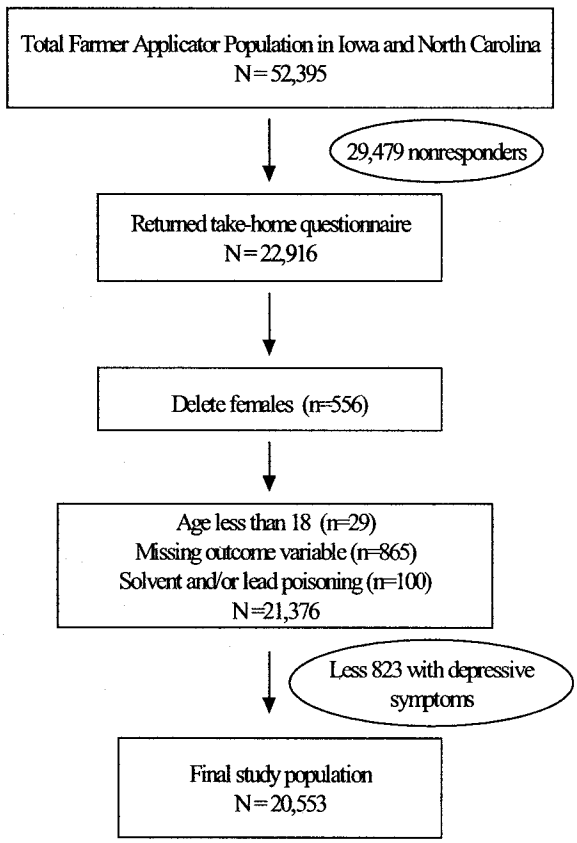
RESULTS

A total of 52,395 farmers in Iowa and North Carolina were enrolled in the AHS from 1993 through 1997 upon applying for a pesticide applicator license (Figure 7). Of the total farmer applicators, 22,916 returned the take-home questionnaire containing the outcome variable, diagnosed depression. Of these, 556 were women and were excluded from this study. Additional exclusions included 29 individuals who were less than 18 years of age, 865 who were missing depression status, and 100 who had a solvent or lead poisoning. To reduce potential misclassification, 823 individuals who reported depressive symptoms at least once per week, but had not been diagnosed with depression, were excluded from the control group. A total of 48 cases of depression were lost due to these exclusions. The final study population consisted of 20,553 with 644 cases of diagnosed depression and 19,909 controls (Figure 7).

The 20,553 study participants who returned the take-home questionnaire differed from the non-respondents in that they were more likely to be older, Caucasian, a high school graduate, and married (Table 3.1). They were also more likely to reside in Iowa rather than North Carolina. They differed on behavioral characteristics such as alcohol use and smoking. They did not differ on how often they felt nervous or depressed and thought it related to pesticides, nor were respondents significantly more or less likely to have visited the doctor as a result of using pesticides. Due to the strong age difference

between respondents and non-respondents, age-adjusted significance tests were used in comparing exposure variables (Table 3.2). Even after adjusting for the age effect, which reduced the significance considerably, the respondents had greater pesticide exposure than the non-respondents.

Figure 7: Flowchart showing exclusions from the AHS farm applicator population.



In this study population, 3.1% reported a diagnosed depression, and this was very similar in North Carolina (2.9%) and Iowa (3.2%). The prevalence of pesticide poisoning

was 1.9% in each state, 131 in North Carolina and 262 in Iowa; 52 had missing information on pesticide poisoning.

The numbers and percents of study participants with various medical conditions and the results of the logistic regression predicting being diagnosed with depression when a medical condition, excluding depression, was present is shown in Table 3.3. The 37 medical conditions resulted in 17 significant associations between a self-reported diagnosed medical condition and diagnosed depression. High blood pressure and pneumonia were the most prevalent conditions reported, but multiple sclerosis and tuberculosis had the highest odds ratio for being diagnosed with depression. This analysis was the basis for calculating the propensity score.

Demographic characteristics were compared between cases and controls (Table 3.4). The race categories had to be collapsed into white and non-white since the study population was greater than 98% Caucasian. There were 185 Hispanics. Cases and controls did not differ significantly by state of residence, race or Hispanic ethnicity. Due to the curvilinear association of age with diagnosed depression, age was categorized based on quartiles of the continuous variable and those under 40 were the reference group in this study. Cases were more likely to be greater than 40 years of age and controls more likely to be less than 40. In addition, cases were less likely to be a high school graduate, less likely to be married, and more likely to abstain from alcohol but be a past or current smoker. Cases were more likely to have visited a doctor and had a propensity score greater than the median.

Farm size, type of livestock raised and crops grown were similar between cases and controls (Table 3.5). Those who worked off the farm and those who lived on farms

without crops or animals showed significantly elevated odds of being depressed. No significant differences were seen in the years during their lifetime they had lived or worked on a farm. The only significant difference in farm products was that controls were more likely than cases to grow cotton and peanuts.

Table 3.6 compares exposure histories in cases and controls using univariate logistic regression. Personally mixing or applying pesticides did not differ between controls and cases, although cases reported a slightly greater number of years of mixing and applying pesticides. Exposure time differed between cases and controls (WMW chi-square 6.71, $p=0.0096$). Exposure time also differed between those with a pesticide poisoning and those without a poisoning (WMW chi-square 75.2, $p<0.0001$). Farmer applicators who had an unusually high exposure or a diagnosed poisoning were more likely to fall into the higher categories of lifetime exposure than those who did not (chi-square for trend 264.4; $p <0.0001$ for unusually high exposure and chi-square for trend 85.6; $p<0.0001$ for a poisoning). In this study, those with diagnosed depression and/or a diagnosed pesticide poisoning reported greater total lifetime exposure to pesticides.

The use of personal protective equipment did not differ between cases and controls. Solvent exposure was significantly associated with depression. Exposure levels showed a significant dose-response relationship, with the reference group being those who personally handled pesticides fewer than 225 days (the lowest quartile of the exposure time variable). The high exposure level representing chronic exposure without an unusually high exposure or acute poisoning was significant (OR 1.44; CI 1.11, 1.86). The unusually high exposure group had a significantly elevated odds ratio of 1.99 (CI 1.60, 2.49), but the acutely poisoned group showed an even larger odds ratio of 4.19 (CI

2.93, 5.99). Greater than 97% of cases and controls reported using herbicides and greater than 92% reported using crop or animal insecticides. Both the use of insecticides, and specifically the use of OP insecticides, significantly differed between cases and controls.

The multivariable analysis was done using two separate methods of adjusting for medical conditions (Table 3.7). Although state of residence was not significant in the univariate analysis, farmers living in Iowa were more likely to be diagnosed with depression than farmers in North Carolina, regardless of how medical status was adjusted. Being unmarried increased the odds of being diagnosed with depression. Drinking alcohol monthly or less or being a past or current smoker increased the odds of being diagnosed with depression. Producing crops or livestock was protective against being diagnosed with depression. Solvent exposure showed a significantly elevated odds ratio in both models. The four categories of pesticide exposure showed a dose-response relationship, with a doubling of the odds ratio with having reported a diagnosed poisoning compared to having an unusually high exposure. After adjusting for all of the above confounding variables, pesticide exposure remained strongly associated with depression. Greater than 752 days of exposure was significant, as were both having an unusually high exposure and a diagnosed poisoning. A slightly greater association was observed between poisoning and depression when adjusting for doctor visits than when adjusting for both doctor visits and propensity score. Examining the likelihood ratio statistics of each model showed that the data fit the model better when using only doctor visits rather than the combined doctor visits and propensity score.

Stratifying by the number of doctor visits in the past year and running a stepwise logistic regression of the above variables revealed a pattern in the exposure variables

(Table 3.8). Those who were presumably healthy and did not see the doctor showed a much higher odds ratio for diagnosed poisoning and depression than those who did visit the doctor. Only having an unusually high exposure or diagnosed poisoning were statistically significant in those who did not see a doctor. In those who did visit the doctor, diagnosed depression remained significantly associated with poisoning, but became less significant as other factors became more significant, including having a propensity score above the median.

Table 3.9 shows six different models stratifying by state of residence. The same dose-response relationships can be seen by state, with North Carolina farmers having higher odds ratios for pesticide poisoning and depression than Iowa farmers. Models five and six showed that adjusting for both doctor visits and propensity score increased the odds ratio associated with poisoning and depression in North Carolina, whereas in Iowa the odds ratio for poisoning and depression was reduced.

Table 3.10 describes pesticide symptoms by exposure level and examines six symptoms in both univariate and multivariable models of depression. Due to small numbers, the low and moderate exposure levels were collapsed into 0 to 752 days. With the exception of nausea or vomiting, symptoms experienced by exposure level showed a dose-response relationship, with diagnosed poisoning showing the strongest associations.

Those who reported at least sometimes having at least two symptoms were excluded from the analysis if they did not report an unusually high exposure or poisoning (Table 3.11). This model was run using doctor visits to adjust for medical/diagnosis status. The results shown in Table 3.11 are similar to those in Table 3.7, except that the association with depression in those in the lower exposure categories became

insignificant while those with an unusually high exposure or pesticide poisoning increased.

DISCUSSION

Those who returned the take-home questionnaire differed in demographic, behavioral and pesticides exposure characteristics from those who did not. Therefore, the subset of the AHS population who returned the take-home questionnaire is not representative of the entire AHS population. Important to this study, however, is that respondents did not differ from non-respondents in regard to the frequency of nervousness or depression they thought related to pesticides, nor did either group preferentially seek a doctor due to pesticide use. Exposure levels were higher in respondents than non-respondents, providing an adequate range of exposure for analysis and making it unlikely that exposures were underestimated in this AHS population.

Overall, the rate of diagnosed depression (3.1%) was relatively low in this farming population. This could be due to using diagnosed depression as the outcome as opposed to a scale measuring depressive symptoms, or it could be that farm residents are not likely to seek treatment for medical conditions, as has been observed in other studies in farming populations. Linn and Husaini (1987) reported that only 5.3% of farmers who were depressed using the CES-D scale sought medical treatment.

Where the number of diseases was of sufficient size to test, medical conditions were strongly and significantly associated with the number of doctor visits in this study. Using doctor visits in the past year and propensity score based on the median of 2% showed that seeing a doctor fewer than two times was protective against being diagnosed with depression, even when above the median propensity score. Seeing a doctor at least

two times showed twice the odds of being diagnosed with depression than only one visit. Model fit parameters suggested that using doctor visits was a better fit to the data than using doctor visits with propensity score. From this analysis, it appears that the probability of a doctor diagnosing depression increases with the number of doctor-patient interactions.

The number of medical conditions present was statistically significantly dissimilar across categories of doctor visits and pesticide exposure level (Jonckheere-Terpstra test for ordered differences, Z=6.39, 2-sided p-value <0.0001). This suggests that an individual's medical conditions were associated with pesticide exposure level and number of doctor visits. To further explore the relationship between diagnosed depression and pesticide exposure, the analysis was stratified by number of doctor visits. An unusually high exposure and poisoning showed greater significance in those who did not see a doctor and the association weakened with increased doctor visits. Other factors became significant in those with one or more visits to the doctor. It is likely that doctor visits represents having a long-standing medical condition or serious injury or illness and the doctor diagnosing depression after several visits. Doctor visits appear to be adequate for adjusting for possible confounding related to health status in this farming population. Given the results of this analysis, confounding by unadjusted health effects is unlikely to have resulted in the significant findings in this study.

After adjusting for significant covariates, exposures of greater than 752 days, an unusually high exposure and a poisoning remained significant, but the acute poisoning showed a two-fold increase over an unusually high exposure, regardless of how medical conditions and doctor visits were adjusted for. This result held in multivariable models

after stratifying by state where North Carolina was shown to have an even greater association of pesticide poisoning with depression. After excluding the farmers who had symptoms but not an unusually high exposure or poisoning, the greater than 752 days category became insignificant and the unusually high and poisoning categories became more significant. It is unlikely that these farmers were experiencing a very high exposure with every pesticide application and it is more likely attributable to having been sensitized to pesticides and experiencing multiple chemical sensitivity (MCS). Since MCS is associated with depression, it makes sense that this result showed significance at nearly every level of pesticide exposure, and because it is thought to result from a very high exposure to OPs and carbamates, it makes sense that the highest exposure levels showed a greater association. It could also be a marker for genetic susceptibility in some individuals at lower exposure levels, as has been demonstrated in the ability to breed rats that are sensitive to OP compounds (Russell et al. 1987).

The dose-response relationship of symptoms related to OP and carbamate use may provide indirect evidence that these chemicals were responsible for the association with depression. The increase of 20.5% in those with an unusually high exposure and 17.2% in those with a poisoning suggests that exposure was misclassified and these farmers may have experienced a very high exposure in the past. It also suggests that future studies should ask study participants about symptoms experienced when using pesticides and a history of poisoning should be sought. These findings are supported by previous work where symptoms of anxiety and depression were reported in pesticide-poisoned individuals (Reidy et al. 1992; Stokes et al. 1995). Stokes et al (1995) did not ask about past poisoning, but did have individuals who reported symptoms, and they saw

elevated associations with neurological effects and pesticide exposure. Reidy et al (1992) study subjects experienced symptoms after a poisoning and had greater anxiety and depression.

Although the results were not shown here, an analysis was conducted to determine whether including work practices, which influence exposure, would have altered these results. Exposure time was multiplied by the intensity score from an algorithm published by Dosemeci et al. (2002) for each type of pesticide including crop insecticides, animal insecticides, herbicides, fungicides and fumigants. The intensity score used the variables for mixing, the mixing system, application method, the tractor type, equipment repair, washing of pesticide equipment, personal protective equipment worn and personal hygiene (Dosemeci et al. 2002). Replacing the exposure time categories with the intensity measure and running a multivariable analysis identified no differences between using exposure time and using exposure time multiplied by the intensity score.

Studies using neurological testing are subject to a plethora of influencing factors such as age, education, language preference, the presence of other neurological conditions, caffeine consumption, alcohol and other drug use, fatigue and sleep patterns and day to day variation in individual alertness. Having depressive symptoms and mood changes might be a much simpler marker of an underlying neurological condition and just as reliable as the battery of neurological tests that have been performed on pesticide-exposed individuals. A review of the literature shows that whenever neurological effects are observed and mood is examined, they tend to coincide (Farahat et al. 2003; Jamal et al. 2002; Stephens et al. 1995). Physicians should be alert to depression and anxiety in their patients following a high pesticide exposure event. Further, future work should

address the relationship of persistent symptoms when using pesticides and determine whether this condition is related to other neurological conditions previously reported in the literature. Studies on the relationship of MCS and depression and whether it is related to neuropathy would be beneficial because depression is an important issue in the quality of life and can be highly disabling, resulting in a greater risk of disease and psychiatric morbidity. It is possible that all these conditions are connected through an underlying alteration in the cholinergic system.

A limitation of this study is that reported age at diagnosis of depression and age at pesticide poisoning were asked in 20-year age categories. The literature shows that the effects of a poisoning can last for many years (Reidy et al. 1992; Savage et al. 1988; Steenland et al. 1994). This makes the temporal relationship impossible to assess, as is true for case-control studies in general. The causal pathway might be depression leading to the higher probability of an unusually high pesticide exposure or pesticide poisoning. Additionally, data on confounding factors such as financial information, including income and debt, and indicators of social support were not available. These factors could be related to stress in the farmers, which may increase the probability of having a pesticide poisoning and may also result in depression.

Table 3.1. Description and comparison of those who did not return the take-home questionnaire and those who did using chi-square tests (N=49,135), Agricultural Health Study, 1993-1997.

Variable	Not returned (N=28,582) n (%)	Returned (N=20,553) n (%)	χ^2	p-value
State of Residence				
Iowa	16803 (58.8)	13632 (66.3)		
North Carolina	11779 (41.2)	6921 (33.7)	288	<0.0001
Nonwhite race	1034 (3.7)	328 (1.6)	180	<0.0001
Hispanic ethnicity	303 (1.2)	185 (1.0)	4.36	0.0367
Age				
< 40	10338 (36.2)	5423 (26.4)		
40-49	7402 (25.9)	4832 (23.5)		
50-59	5799 (20.3)	4778 (23.2)		
> 59	5043 (17.6)	5520 (26.9)	900*	<0.0001
High school graduate	24437 (89.2)	17920 (91.1)	45.5	<0.0001
Married	23829 (83.7)	17368 (84.9)	11.2	0.0008
Alcohol Use				
Never	8505 (32.3)	6897 (35.3)		
< 3 per month	8353 (31.7)	6439 (32.9)		
1 – 4 per week	7856 (29.8)	5025 (25.7)		
Every /almost every day	1627 (6.2)	1193 (6.1)	103*	<0.0001
Cigarette smoking				
Never	14439 (52.3)	10792 (54.2)		
Past	8125 (29.5)	6564 (33.0)		
Current	5027 (18.2)	2538 (12.8)	272	<0.0001
How often felt nervous or depressed and related to using pesticides				
Never/Rarely	24244 (90.5)	17605 (91.0)		
Sometimes	2386 (8.9)	1665 (8.2)		
Frequently/Almost Always	151 (0.6)	83 (0.4)	5.47	0.0648
Visited doctor as a result of using pesticides:				
Never	25803 (93.8)	18542 (93.4)		
Once or more often	1711 (6.2)	1305 (6.6)	2.46	0.1168

* Mantel-Haenszel statistic for trend.

Table 3.2. Numbers, percents and age-adjusted chi-square tests comparing pesticide exposure between those who did and did not return the take-home questionnaire in the AHS, 1993-1997.

Variable	Not Returned (N=28,582) n (%)	Returned (N=20,553) n (%)	$\chi 2$	p-value
Personally mixed or applied pesticides	27663 (99.0)	19927 (99.5)	23.2	<0.0001
% of time personally mixed pesticides				
Never	1211 (4.6)	744 (3.9)		
< 50%	6100 (23.1)	4368 (22.9)		
50% or more	19074 (72.3)	14000 (73.2)	4.26*	0.0391
% of time personally applied pesticides				
Never	730 (2.8)	370 (1.9)		
< 50%	5423 (20.6)	3720 (19.5)		
50% or more	20226 (76.7)	15005 (78.6)	14.3*	0.0002
Days per year applied pesticides				
< 5 days	4794 (18.1)	3591 (18.8)		
5 – 9 days	5915 (22.4)	4792 (25.0)		
10 – 19 days	7704 (29.1)	5914 (30.9)		
20 – 39 days	5364 (20.3)	3421 (17.9)		
40 – 59 days	1467 (5.5)	843 (4.4)		
60 – 150 days	974 (3.7)	507 (2.7)		
> 150 days	239 (0.9)	89 (0.5)	33.9*	<0.0001
Years personally applied or mixed				
1 year or less	904 (3.4)	433 (2.3)		
2 – 5 years	3176 (11.9)	1881 (9.8)		
6 – 10 years	4278 (16.0)	2720 (14.1)		
11 – 20 years	8960 (33.6)	6286 (32.6)		
21 – 30 years	6271 (23.5)	4920 (25.5)		
> 30 years	3091 (11.6)	3031 (15.7)	34.1*	<0.0001

*Mantel-Haenszel statistic for trend.

Table 3.3. Odds ratios and 95% confidence intervals for being diagnosed with depression (n=644) when a different medical condition is present in 20,548 farmers, AHS 1994-1996.*

Characteristic	n (%)	OR	95% CI
Angina	1078 (5.2)	1.88	1.45, 2.44
Arrhythmia	1254 (6.1)	1.54	1.19, 2.00
Asthma	996 (4.9)	1.41	1.05, 1.89
Bronchitis	780 (3.8)	2.28	1.71, 3.03
Eczema	498 (2.4)	1.53	1.03, 2.27
Epilepsy	134 (0.7)	2.85	1.57, 5.15
Farmer's lung	441 (2.2)	1.53	1.03, 2.27
Traumatic brain injury	2456 (12.0)	1.26	1.01, 1.57
High blood pressure	3406 (16.6)	1.62	1.34, 1.95
Mononucleosis	1018 (5.0)	1.47	1.07, 2.03
Multiple Sclerosis	29 (0.1)	17.5	7.48, 41.1
Pneumonia	2994 (14.6)	1.33	1.09, 1.63
Rheumatoid arthritis	1141 (5.6)	1.39	1.05, 1.83
Grave's disease	93 (0.5)	0.16	0.03, 0.75
Thyroid disease	247 (1.2)	2.03	1.23, 3.35
Colitis	216 (1.1)	2.12	1.26, 3.58
Tuberculosis	56 (0.3)	3.12	1.30, 7.50

* Five individuals were missing information on medical conditions.

Table 3.4. Demographic differences between cases (n=644) and controls (n=19,909) in 20,553 Iowa and North Carolina farmer pesticide applicators, 1993-1997.

Characteristic	Cases n (%)	Controls n (%)	χ^2 (p-value)	OR (95% C.I.)
State of residence				
Iowa	443 (68.8)	13189 (66.3)	reference	reference
North Carolina	201 (31.2)	6720 (33.7)	1.81 (0.1791)	0.89 (0.75, 1.05)
Race				
White	620 (99.0)	19048 (98.3)	reference	reference
Nonwhite	6 (1.0)	322 (1.7)	1.86 (0.1724)	0.57 (0.25, 1.29)
Hispanic ethnicity	4 (0.7)	181 (1.0)	0.57 (0.4512)	0.68 (0.25, 1.85)
Age quartiles				
< 40	107 (16.6)	5316 (26.7)	reference	reference
40 – 49	197 (30.6)	5088 (25.6)	28.9 (<0.0001)	1.92 (1.52, 2.44)
50 – 59	193 (30.0)	4639 (23.3)	35.3 (<0.0001)	2.07 (1.63, 2.63)
> 59	147 (22.8)	4866 (24.4)	10.0 (0.0016)	1.50 (1.17, 1.93)
< High school education	544 (88.7)	17376 (91.2)	4.31 (0.0380)	1.31 (1.01, 1.69)
Unmarried	527 (82.0)	16841 (84.9)	4.34 (0.0372)	1.24 (1.01, 1.53)
Doctor visits past year				
None	94 (14.7)	7076 (35.9)	reference	reference
Once	160 (25.1)	6264 (31.7)	24.9 (<0.0001)	1.92 (1.49, 2.49)
More than once	384 (60.2)	6391 (32.4)	168 (<0.0001)	4.52 (3.60, 5.68)
Propensity score				
≤ median	300 (46.6)	13994 (70.3)	reference	reference
> median	344 (53.4)	5910 (29.7)	166 (<0.0001)	2.72 (2.32, 3.18)
Visits + propensity score				
≤ median (2%)	298 (46.7)	13871 (70.3)	reference	reference
> median/0 or 1 visit	92 (14.4)	2631 (13.3)	16.2 (<0.0001)	1.63 (1.28, 2.06)
> median/ 2+ visits	248 (38.9)	3224 (16.3)	209 (<0.0001)	3.58 (3.01, 4.26)
Alcohol Use				
Never	235 (38.3)	6662 (35.2)	6.88 (0.0087)	1.34 (1.08, 1.66)
< 3 per month	210 (34.3)	6229 (32.9)	4.71 (0.0299)	1.28 (1.02, 1.60)
1 – 4 per week	129 (21.0)	4896 (25.8)	reference	reference
Every /almost every day	39 (6.4)	1154 (6.1)	1.80 (0.1802)	1.28 (0.89, 1.85)
Cigarette smoking				
Never	296 (47.4)	10496 (54.5)	reference	reference
Past	241 (38.6)	6323 (32.8)	11.7 (0.0006)	1.35 (1.14,1.61)
Current	87 (13.9)	2451 (12.7)	3.44 (0.0635)	1.26 (0.99, 1.61)

Table 3.5. Odds ratios and 95% confidence intervals for differences in farm characteristics between 20,553 cases and controls, AHS, 1993-1997.

Farm Characteristic	Cases (n=644) n (%)	Controls (n=19,909) n (%)	OR (C.I.)
Do not work on the farm	20 (3.1)	442 (2.2)	1.41 (0.90, 2.23)
Worked at a job off the farm	444 (69.3)	12785 (64.8)	1.23 (1.03, 1.45)
Farms with no crops or animals	13 (2.0)	169 (0.9)	2.41 (1.36, 4.25)
No. of years worked on farm			
< 5	12 (1.9)	244 (1.2)	
5 – 10	12 (1.9)	378 (1.9)	
11 – 20	44 (6.9)	1360 (6.9)	
21 – 30	72 (11.3)	2672 (13.6)	
> 30	496 (78.0)	14946 (76.3)	1.00 (0.91, 1.10)
Farm Products			
Cattle or hogs	113 (17.6)	3710 (18.6)	0.96 (0.87, 1.07)
Sheep/chickens	14 (2.2)	298 (1.5)	1.10 (0.90, 1.35)
Fruit	24 (3.7)	480 (2.4)	1.07 (0.94, 1.22)
Vegetables	82 (12.7)	2663 (13.4)	1.00 (0.93, 1.08)
Grains	178 (27.6)	5514 (27.7)	1.01 (0.92, 1.11)
Alfalfa/hay	255 (39.6)	7890 (39.6)	1.00 (0.85, 1.17)
Christmas trees	17 (2.6)	488 (2.4)	1.08 (0.66, 1.76)
Cotton	13 (2.0)	722 (3.6)	0.55 (0.32, 0.95)
Peanuts	13 (2.0)	728 (3.7)	0.54 (0.31, 0.95)
Tobacco	62 (9.6)	2445 (12.3)	0.76 (0.58, 0.99)
Number of acres planted			
none	24 (4.1)	618 (3.4)	
< 5 acres	19 (3.2)	690 (3.8)	
5 – 49 acres	53 (9.1)	1770 (9.7)	
50 – 199 acres	115 (19.7)	3342 (18.3)	
200 – 499 acres	184 (31.5)	5295 (29.0)	
500 – 999 acres	132 (22.6)	4064 (22.2)	
> 1,000 acres	57 (9.8)	2483 (13.6)	0.96 (0.91, 1.01)
Number of poultry			
none	517 (90.2)	16103 (90.4)	
< 50	27 (4.7)	846 (4.8)	
50 – 99	14 (2.4)	221 (1.2)	
100 – 499	7 (1.2)	223 (1.2)	
500 – 999	2 (0.4)	31 (0.2)	
1,000 – 10,000	0 (0.0)	53 (0.3)	
> 10,000	6 (1.1)	340 (1.9)	0.94 (0.86, 1.04)
Number of livestock			
none	188 (30.8)	5953 (32.9)	
< 50	75 (12.3)	2527 (14.0)	
50 – 99	42 (6.9)	1454 (8.1)	
100 – 499	119 (19.5)	3595 (19.9)	
500 – 999	61 (10.0)	2189 (12.0)	
>1,000	96 (15.8)	2349 (13.0)	1.01 (0.97, 1.06)

Table 3.6. Numbers, percents, chi-square test statistics and odds ratios from univariate logistic regression analyses comparing chemical exposures between 644 cases and 19,909 controls in 20,553 farmer applicators in the AHS, 1993-1997.

Variable	Cases n (%)	Controls n (%)	$\chi 2$ (p-value)	OR 95% CI
Personal protective equipment				
Used	584 (90.7)	18248 (91.7)	reference	reference
Not used	60 (9.3)	1659 (8.3)	0.79 (0.3754)	1.13 (0.86, 1.48)
Solvent exposure				
No	512 (79.5)	16666 (83.7)	reference	reference
Yes	132 (20.5)	3243 (16.3)	8.02 (0.0046)	1.33 (1.09, 1.61)
% of time mixed pesticides				
Never	22 (3.6)	722 (3.9)	reference	reference
< 50%	109 (18.0)	4259 (23.0)	0.54 (0.4628)	0.84 (0.53, 1.34)
50% or more	476 (78.4)	13524 (73.1)	0.42 (0.5149)	1.16 (0.75, 1.78)
% of time applied pesticides				
Never	9 (1.5)	361 (2.0)	reference	reference
< 50%	95 (15.7)	3625 (19.6)	0.02 (0.8881)	1.05 (0.53, 2.10)
50% or more	502 (82.8)	14503 (78.4)	0.93 (0.3358)	1.39 (0.71, 2.70)
Days per year mixed/applied				
None	1 (0.2)	92 (0.5)		
Less than 5 days	109 (17.9)	3389 (18.3)		
5 – 9 days	160 (26.2)	4632 (25.0)		
10 – 19 days	186 (30.5)	5728 (30.9)		
20 – 39 days	101 (16.6)	3320 (17.9)		
40 – 59 days	30 (4.9)	813 (4.4)		
60 – 150 days	19 (3.1)	488 (2.6)		
More than 150 days	4 (0.7)	85 (0.4)	0.74 (0.3898)	1.00 (1.00, 1.01)
Years mixed/applied				
None	1 (0.2)	92 (0.5)		
1 year or less	12 (2.0)	328 (1.8)		
2 – 5 years	32 (5.3)	1849 (9.9)		
6 – 10 years	68 (11.2)	2652 (14.2)		
11 – 20 years	193 (31.7)	6093 (32.6)		
21 – 30 years	188 (30.9)	4732 (25.4)		
30 + years	114 (18.7)	2917 (15.6)	22.1 (<0.0001)	1.02 (1.01, 1.03)
Exposure levels *				
0 – 224 days	246 (40.3)	9629 (51.7)	reference	reference
225 – 752 days	125 (20.5)	4067 (21.9)	2.75 (0.0971)	1.20 (0.97, 1.50)
> 752 days	79 (12.9)	2149 (11.5)	7.66 (0.0057)	1.44 (1.11, 1.86)
Unusually high	123 (20.1)	2416 (13.0)	37.4 (<0.0001)	1.99 (1.60, 2.49)
Diagnosed poisoning	38 (6.2)	355 (1.9)	61.7 (<0.0001)	4.19 (2.93, 5.99)
Used herbicides	633 (98.3)	19319 (97.0)	3.37 (0.0662)	1.76 (0.96, 3.21)
Used insecticides	615 (95.5)	18298 (91.9)	10.6 (0.0011)	1.87 (1.28, 2.72)
Used OPs	587 (91.2)	17190 (86.3)	12.1 (0.0005)	1.63 (1.24, 2.14)
Used fungicides	223 (34.6)	6558 (32.9)	0.80 (0.3702)	1.08 (0.91, 1.27)
Used fumigants	150 (23.3)	4118 (20.7)	2.57 (0.1086)	1.16 (0.97, 1.40)

* Exposure time <= median of 224 days is reference group; 225 to 752 is median to 90th percentile; > 752 is above the 90th percentile; reporting an unusually high exposure regardless of exposure time represents level 4; the highest level is reporting a diagnosed pesticide poisoning. Trend test over exposure time categories not significant (chi-square 3.12; p=0.0773).

Table 3.7. Multivariate logistic regression analysis of exposure levels and diagnosed depression adjusting for demographic and farm characteristics shown to be significant in the univariate analysis in 20,553 cases and controls using doctor visits and doctor visits with median propensity score, AHS, 1993-1997.

Variable	OR (95% CI) Using doctor visits (574 cases /17475 controls)	OR (95% CI) Doctor visits+propensity score (576 cases/17471 controls)
State of residence		
Iowa	reference	reference
North Carolina	0.69 (0.56, 0.85)	0.71 (0.57, 0.87)
Age		
< 40	reference	reference
40 – 49	1.65 (1.28, 2.14)	1.65 (1.28, 2.14)
50 – 59	1.65 (1.26, 2.16)	1.59 (1.21, 2.09)
> 59	0.93 (0.69, 1.25)	0.92 (0.68, 1.25)
< High school education	1.46 (1.09, 1.95)	1.42 (1.06, 1.90)
Unmarried	1.82 (1.45, 2.28)	1.72 (1.37, 2.16)
Visits to doctor in past year		
None	reference	
Once	1.86 (1.42, 2.44)	
More than once	4.42 (3.47, 5.65)	
Doctor visits and propensity score		
≤ median (2%)		reference
> median/0 or 1 visit		1.47 (1.14, 1.90)
> median/ 2+ visits		3.47 (2.86, 4.22)
Alcohol Use		
Never	1.46 (1.14, 1.86)	1.49 (1.16, 1.90)
< 3 per month	1.29 (1.02, 1.63)	1.33 (1.06, 1.68)
1 – 4 per week	reference	reference
Every /almost every day	1.17 (0.80, 1.71)	1.16 (0.79, 1.70)
Smoking		
Never smoker	reference	reference
Past smoker	1.26 (1.04, 1.52)	1.25 (1.03, 1.51)
Current smoker	1.33 (1.02, 1.74)	1.25 (0.96, 1.64)
Do not have crops/livestock	2.35 (1.20, 4.61)	2.33 (1.18, 4.59)
Work job off farm	1.19 (0.97, 1.45)	1.19 (0.98, 1.46)
Solvent exposure	1.28 (1.03, 1.60)	1.26 (1.01, 1.57)
Exposure levels *		
0 – 224 days	reference	reference
225 – 752 days	1.21 (0.96, 1.53)	1.20 (0.95, 1.51)
> 752 days	1.46 (1.11, 1.91)	1.47 (1.12, 1.92)
Unusually high	1.95 (1.55, 2.45)	1.97 (1.56, 2.48)
Diagnosed poisoning	3.83 (2.63, 5.60)	3.70 (2.53, 5.41)

* Exposure time <= median of 224 days is reference group; 225 to 752 is median to 90th percentile; > 752 is above the 90th percentile; reporting an unusually high exposure regardless of exposure time represents level 4; the highest level is reporting a diagnosed pesticide poisoning.

Table 3.8. Multivariate logistic regression results from a stepwise* analysis of exposure levels and diagnosed depression stratified by number of visits to a physician in the past twelve months, AHS, 1993-1997.

Models stratified by visits to doctor	Chi-square (p-value)	OR (95% CI)
Model 1: No doctor visits in past year		
88 cases and 6296 controls		
Unusually high pesticide exposure	8.62 (0.0033)	2.17 (1.29, 3.65)
Diagnosed pesticide poisoning	13.0 (0.0003)	5.63 (2.20, 14.4)
Model 2: One visit to doctor in past year		
143 cases and 5565 controls		
Married	6.77 (0.0093)	1.73 (1.15, 2.62)
< High school education	4.04 (0.0444)	1.77 (1.01, 3.09)
Unusually high pesticide exposure	9.37 (0.0022)	1.90 (1.26, 2.87)
Diagnosed pesticide poisoning	6.49 (0.0108)	2.83 (1.27, 6.32)
40 – 49 years of age	9.81 (0.0017)	1.74 (1.23, 2.46)
Propensity score > median of 2	20.2 (<0.0001)	2.18 (1.55, 3.06)
Model 3: 2+ visits to doctor in past year		
345 cases and 5610 controls		
Married	21.9 (<0.0001)	2.04 (1.51, 2.74)
Solvent exposure	5.78 (0.0162)	1.39 (1.06, 1.82)
Unusually high pesticide exposure	11.5 (0.0007)	1.61 (1.22, 2.13)
Diagnosed pesticide poisoning	21.5 (<0.0001)	2.96 (1.87, 4.67)
40 – 49 years old	13.0 (0.0003)	1.68 (1.27, 2.23)
50 – 59 years old	24.6 (<0.0001)	1.91 (1.48, 2.47)
Propensity score > median of 2	24.6 (<0.0001)	1.79 (1.42, 2.25)

*Covariates included in the model were state of residence, age, education, marital status, frequency of alcohol use, past or current smoking, do not have crops or animals, work a job off the farm, solvent exposure, pesticide exposure levels, and below or above the median propensity score.

Table 3.9. Multivariate logistic regression analysis of exposure levels and diagnosed depression adjusting for demographic and farm characteristics comparing Iowa and North Carolina farmer applicators, AHS, 1993-1997.

Variable	Iowa OR (95% CI)	North Carolina OR (95% CI)
Model 1: Exposure:	Cases n=433; Controls 12679	Cases n=178; Controls n=5937
0 – 224 days	reference	reference
225 – 752 days	1.31 (1.02, 1.69)	0.96 (0.62, 1.47)
> 752 days	1.50 (1.07, 2.09)	1.38 (0.92, 2.08)
Unusually high	2.09 (1.62, 2.69)	1.68 (1.06, 2.66)
Diagnosed poisoning	4.10 (2.65, 6.37)	4.38 (2.36, 8.11)
Model 2: Exposure:	Cases n=433; Controls=12679	Cases n=178; Controls n=5937
0 – 224 days	reference	reference
225 – 752 days	1.21 (0.94, 1.57)	0.96 (0.62, 1.47)
> 752 days	1.31 (0.93, 1.84)	1.38 (0.91, 2.07)
Unusually high	2.03 (1.57, 2.62)	1.75 (1.10, 2.78)
Diagnosed poisoning	3.85 (2.47, 5.99)	4.36 (2.35, 8.08)
Model 3: Exposure:	Cases n=429; Controls n=12608	Cases n=176; Controls n=5855
0 – 224 days	reference	reference
225 – 752 days	1.19 (0.92, 1.55)	0.99 (0.64, 1.52)
> 752 days	1.28 (0.91, 1.80)	1.36 (0.90, 2.05)
Unusually high	1.81 (1.40, 2.35)	1.68 (1.05, 2.69)
Diagnosed poisoning	3.28 (2.08, 5.17)	3.66 (1.95, 6.85)
Model 4: Exposure:	Cases n=429; Controls n=12604	Cases n=176; Controls n=5855
0 – 224 days	Reference	reference
225 – 752 days	1.19 (0.92, 1.54)	0.97 (0.63, 1.50)
> 752 days	1.26 (0.89, 1.77)	1.41 (0.93, 2.13)
Unusually high	1.84 (1.42, 2.38)	1.67 (1.04, 2.68)
Diagnosed poisoning	3.05 (1.93, 4.82)	3.71 (1.97, 6.96)
Model 5: Exposure:	Cases n=414; Controls n=12149	Cases n=162; Controls n=5326
0 – 224 days	reference	reference
225 – 752 days	1.26 (0.96, 1.65)	1.08 (0.68, 1.72)
> 752 days	1.36 (0.95, 1.93)	1.69 (1.09, 2.61)
Unusually high	1.91 (1.47, 2.49)	2.12 (1.30, 3.45)
Diagnosed poisoning	3.64 (2.29, 5.77)	4.29 (2.19, 8.40)
Model 6: Exposure:	Cases n=414; Controls n=12145	Cases n=162; Controls n=5326
0 – 224 days	reference	reference
225 – 752 days	1.26 (0.96, 1.64)	1.06 (0.67, 1.68)
> 752 days	1.34 (0.94, 1.91)	1.71 (1.10, 2.65)
Unusually high	1.93 (1.48, 2.51)	2.11 (1.29, 3.43)
Diagnosed poisoning	3.40 (2.14, 5.41)	4.64 (2.38, 9.06)

* Model 1, unadjusted; Model 2, age adjusted; Model 3, adjusted by age and visits to a doctor; Model 4, adjusted by age and combined doctor visits and propensity score; Model 5, adjusted by age, doctor visits and variables in Table 7; Model 6, adjusted by age, combined doctor visits and propensity score and variables in Table 7.

Table 3.10. Descriptive and univariate and multivariate logistic regression models stratifying exposure level by symptoms characteristic of organophosphate and carbamate exposure in 644 cases and 19,909 controls, AHS, 1993-1997.

Pesticide Symptom by Exposure Level	n (%)	Univariate Models OR (95% CI)	Multivariate Models * OR (95% CI)
Excessively tired			
No symptoms	16874 (87.2)	reference	reference
0 – 752 days	954 (4.9)	1.61 (1.16, 2.23)	1.58 (1.12, 2.23)
> 752 days	871 (4.5)	2.27 (1.69, 3.05)	2.18 (1.60, 2.97)
Unusually high	527 (2.7)	3.02 (2.17, 4.21)	2.61 (1.85, 3.67)
Diagnosed poisoning	134 (0.7)	5.92 (3.61, 9.69)	4.92 (2.92, 8.31)
Headaches/dizziness			
No symptoms	14500 (74.6)	reference	reference
0 – 752 days	2011 (10.3)	1.63 (1.28, 2.07)	1.60 (1.23, 2.07)
> 752 days	1721 (8.9)	1.91 (1.50, 2.44)	1.89 (1.46, 2.45)
Unusually high	992 (5.1)	2.59 (1.97, 3.41)	2.39 (1.80, 3.18)
Diagnosed poisoning	205 (1.1)	3.91 (2.41, 6.33)	3.56 (2.17, 5.86)
Nausea or vomiting			
No symptoms	18212 (94.3)	reference	reference
0 – 752 days	359 (1.9)	2.40 (1.57, 3.65)	2.51 (1.61, 3.92)
> 752 days	354 (1.8)	2.98 (2.02, 4.40)	2.85 (1.89, 4.31)
Unusually high	283 (1.5)	2.27 (1.40, 3.69)	2.20 (1.34, 3.60)
Diagnosed poisoning	105 (0.5)	2.76 (1.33, 5.70)	2.52 (1.20, 5.29)
Skin irritation			
No symptoms	15713 (81.3)	reference	reference
0 – 752 days	1353 (7.0)	1.31 (0.97, 1.76)	1.29 (0.95, 1.76)
> 752 days	1368 (7.1)	1.35 (1.01, 1.80)	1.33 (0.98, 1.80)
Unusually high	759 (3.9)	2.30 (1.69, 3.12)	2.07 (1.51, 2.84)
Diagnosed poisoning	140 (0.7)	2.90 (1.56, 5.41)	2.33 (1.20, 4.54)
Eye irritation			
No symptoms	16308 (84.6)	reference	reference
0 – 752 days	1073 (5.6)	1.28 (0.92, 1.79)	1.24 (0.87, 1.76)
> 752 days	1177 (6.1)	1.48 (1.10, 1.99)	1.63 (1.20, 2.23)
Unusually high	623 (3.2)	2.33 (1.67, 3.25)	2.02 (1.43, 2.85)
Diagnosed poisoning	107 (0.5)	5.12 (2.90, 9.05)	4.37 (2.42, 7.89)
Chest discomfort			
No symptoms	17994 (93.2)	reference	reference
0 – 752 days	436 (2.2)	1.88 (1.23, 2.89)	1.78 (1.15, 2.75)
> 752 days	462 (2.4)	2.43 (1.67, 3.53)	2.21 (1.49, 3.28)
Unusually high	323 (1.7)	2.96 (1.96, 4.46)	2.46 (1.62, 3.75)
Diagnosed poisoning	95 (0.5)	4.43 (2.35, 8.35)	3.30 (1.67, 6.51)

*Adjusted for state of residence, age, education, marital status, alcohol, smoking, working off the farm, not having crops or livestock, solvent exposure, and number of visits to physician in past 12 months.

Table 3.11. Multivariate logistic regression analysis of exposure levels and diagnosed depression excluding those who were chronically exposed and reported two or more symptoms when applying pesticides in 447 cases and 15,091 controls, AHS, 1993-1997.**

Variable	Chi-square (p-value)	OR (95% CI)
State of residence		
Iowa	reference	reference
North Carolina	8.31 (0.0039)	0.71 (0.56, 0.90)
Age		
< 40	reference	reference
40 – 49	10.1 (0.0015)	1.64 (1.21, 2.22)
50 – 59	17.0 (<0.0001)	1.93 (1.41, 2.63)
> 59	0.50 (0.4816)	1.13 (0.80, 1.59)
< High school education	4.99 (0.0255)	1.44 (1.05, 1.98)
Unmarried	17.8 (<0.0001)	1.76 (1.35, 2.29)
Visits to doctor in past year		
None	reference	reference
Once	19.5 (<0.0001)	1.98 (1.46, 2.68)
More than once	100 (<0.0001)	4.17 (3.15, 5.51)
Alcohol Use		
Never	5.70 (0.0170)	1.40 (1.06, 1.84)
< 3 per month	1.92 (0.1659)	1.21 (0.93, 1.57)
1 – 4 per week	reference	reference
Every /almost every day	0.34 (0.5574)	1.14 (0.74, 1.75)
Smoking		
Never smoker	reference	reference
Past smoker	3.98 (0.0461)	1.25 (1.00, 1.56)
Current smoker	5.62 (0.0177)	1.44 (1.07, 1.95)
Do not have crops/livestock	4.59 (0.0321)	2.27 (1.07, 4.81)
Work job off farm	2.03 (0.1547)	1.18 (0.94, 1.63)
Solvent exposure	4.73 (0.0296)	1.32 (1.03, 1.69)
Exposure levels *		
0 – 224 days	reference	reference
224 – 752 days	2.33 (0.1268)	1.24 (0.94, 1.63)
> 752 days	1.61 (0.2049)	1.25 (0.89, 1.77)
Unusually high	47.0 (<0.0001)	2.35 (1.84, 3.01)
Diagnosed poisoning	57.8 (<0.0001)	4.49 (3.05, 6.61)

* Exposure time <= median of 224 days is reference group; 225 to 752 is median to 90th percentile; > 752 is above the 90th percentile; reporting an unusually high exposure regardless of exposure time represents level 4; the highest level is reporting a diagnosed pesticide poisoning.

** Same analysis as Table 7, but excluding those who had symptoms but did not report an unusually high exposure or diagnosed pesticide poisoning.

CHAPTER FOUR: CONCLUSION

The findings from the two analyses herein support the findings of past epidemiological studies that a high-level exposure or acute pesticide poisoning is associated with depression. Unlike previous studies, this work had a very large sample size that comes from a well-defined cohort enrolled between 1993 and 1997 and is representative of the farming population who applied restricted-use pesticides in the United States enrolled between 1993 and 1997. A broad range of exposure was tested in relation to depression and the questionnaire included extensive information about work practices and exposure. This study is based on hypothesis-driven research using a specific disease outcome, diagnosed depression. No previous study has selected cases based on the presence of diagnosed depression and addressed whether depressed individuals were more likely to have experienced a high pesticide exposure or acute poisoning. Previous reports have found mood disorders secondary to testing for neuropathies and other neurological symptoms among those exposed to pesticides, but depression was not the primary focus of the studies. Exceptions to this were a cross-sectional study of Colorado farmers by Stallones and Beseler (2002) and a historical cohort study by Amr et al. (1997) of Egyptian formulators and applicators. Both of these studies focused on depression and reported an association with acute pesticide poisoning (Stallones and Beseler 2002) and with long-term, high pesticide exposures (Amr et al. 1997).

The association between high-level pesticide exposure and depression for farm applicators and their spouses was significant in separate analyses by state, in spite of diverse farming practices and farm characteristics. In North Carolina, a stronger

association of pesticide poisoning and depression was found among farm applicators and spouses than in Iowa. This may be due to a lower frequency of PPE use in North Carolina and interactions with pesticides and exposures associated with growing tobacco in North Carolina, a crop not found in Iowa.

Women did participate in farm work, and half of them participated in mixing and applying pesticides. The analysis showed that women who participated less frequently in mixing or applying pesticides had higher odds of depression. This may indicate that women who spent less time doing farm work were more highly stressed and had less time to get the work done than women who worked with pesticides more frequently. Female farm spouses have not been adequately studied, but because they are under a great deal of stress and exposed to a number of chemicals, they may be at increased risk for an affective disorder, particularly if they have experienced a very high pesticide exposure. An OP or carbamate poisoning can increase acetylcholine activity, which in turn can increase HPA axis activity resulting in elevated levels of CRF, ACTH and corticosterone hormones (Risch et al. 1981). Combining this with increased activity in the noradrenergic stress pathways can create very high levels of stress hormones, which may precipitate a depressive episode over time, or cause recurrent depression in those with a history of affective disorders (Yadid et al. 2000).

The farmer applicators with greater than 752 days of exposure showed a slightly elevated, but statistically significant, increase in the odds for depression. The association between diagnosed depression in the presence of a diagnosed pesticide poisoning was much more profound. Individual variability and genetic susceptibility may play a role in the effects of intermediate or unusually high exposure. This exposure level may

represent variations in dose depending on the use of PPE, chemical mixtures, pesticide formulations, and application methods. Another possibility is that higher chronic exposure increases the likelihood of developing a tolerance to OPs and carbamates, resulting in a subsensitivity of muscarinic receptors, but with exposures high enough to have inhibited other enzymes that are potential targets of OPs and carbamates, such as NTE. Alternatively, sensitization may have occurred from a very high exposure and these individuals had been misclassified as not having had an acute poisoning when in fact they may have.

The toxicological literature shows that neuronal targets such as NTE may explain how long-term, chronic neuropsychiatric effects can result from a significant dose of OP pesticides (Brown et al. 1998). The reason for skepticism in the NTE-OPIDN connection has been experiments in animal models showing that a large dose is required to create the 70% inhibition of NTE required to see any physiological response. It has been assumed that, given the rapid hydrolysis of OP compounds by a number of esterase detoxification systems, a sufficient dose would never reach its biological target (Brown et al. 1998). However, these experiments have been performed in rodents and hens and these models may not extrapolate well to humans. For example, the dose required to inhibit NTE may vary by individual AChE and BuChE status, as well as the status of the detoxification pathways. AChE, BuChE and NTE are found in the same areas of the brain and BuChE has been shown to act as a buffer for AChE. If an individual is applying several chemicals, which utilize the same detoxification pathways, and chronic exposure has reduced AChE and BuChE activity, then it is possible that an intermediate or unusually high exposure could result in a dose sufficient to inhibit NTE or some other esterase. If

the individual is sensitive to cholinergic agonists or acetylcholinesterase inhibitors and has a personal or family history of affective disorders, the genetic groundwork could exist for depressive symptoms to occur and persist. Whether both a compromised detoxification system and susceptibility to affective disorders are required should be addressed in future studies.

The OP and carbamate detoxification systems are known to be efficient. Paraoxonase has been shown to detoxify oxons, such as those produced from chlorpyrifos and diazinon. Dichlorvos, chlorpyrifos, and diazinon are the most volatile of the OP pesticides. Inhalation exposure may lead to a higher probability of these chemicals reaching neuronal targets through the olfactory system, compared to dermal exposure where carboxylesterases readily hydrolyze them. Dichlorvos and chlorpyrifos inhibit NTE. Interestingly, DFP not only inhibits NTE, but also causes aging of the enzyme, and is the chemical that was used to produce the cholinergically sensitive genetic rat model of depression. NTE may be associated with chronic depressive symptoms due to changes in signaling pathways and changes in membrane phospholipid metabolism, including choline availability in the cell. Further work on the downstream effects of inhibited and aged NTE and NTE-like enzymes should provide evidence of which neurotransmission pathways may be affected. All of this evidence taken together suggests a link between inhibition of neuronal targets and altered neurotransmitter pathways leading to depression or mood disorders.

The evidence for AChE inhibition playing a role in chronic toxicity comes from studies in developing countries where exposures are higher than in the United States. In these studies, the effects of pesticide exposure are more often associated with mood

disorders in the absence of a poisoning. This is less likely to occur in the United States where exposures tend to be lower due to better use of PPE, more modern mixing and application equipment and applicators who are aware of the hazards and take greater precautions to prevent a poisoning. If esterase status is already reduced in these individuals, it might not take as high of an exposure for the OP to find and inhibit an NTE or NTE-like enzyme, especially if detoxification systems are compromised or a polymorphism makes them genetically susceptible to toxicity.

Results from the AHS spouse study, where only 50% of the study population applied pesticides to crops or animals, suggests the involvement of certain OPs and carbamate compounds. PCA identified unique factors representing patterns of pesticide use in the spouse applicators. The factor that included chlorpyrifos and diazinon was significantly associated with depression in a univariate logistic regression model. No specific chemicals could be identified as associated with depression in the farmer applicator study because farmers used so many chemicals and nearly everyone in the study mixed and applied herbicides and pesticides. However, the results suggested a dose-response relationship of increased exposure with increased depression in those who had symptoms when they mixed or applied pesticides. This effect was greatly increased in those with a pesticide poisoning. The onset of symptoms with subsequent exposure is characteristic of having been sensitized, and this is likely to have occurred after a past high-level exposure to an OP or carbamate pesticide. Removing these farm applicators from the analysis, assuming they may have been misclassified, increased the odds ratio of being depressed in those with an unusually high exposure or an acute pesticide poisoning and reduced the significant association in those exposed greater than 752 days. All of

this evidence taken together suggests that OPs and carbamates may be responsible for the increased risk of depression, that individuals do show some evidence for having been sensitized to these compounds, and that this sensitization appears to be related to the increased odds of depression.

In these studies, no family or personal past history of affective disorders was available from the questionnaire. It would be informative, given the current understanding of depression mechanisms in humans, to know whether those with diagnosed depression had a recurrent dysthymic disorder, or whether they experienced a major depressive episode after the high-level exposure or acute poisoning. The temporal aspects of pesticide poisoning and the development of depressive symptoms have not been addressed in any studies to date, but depression has been reported to persist years after a pesticide poisoning. Epidemiological studies should address this issue by obtaining complete histories of affective disorders prior to the poisoning event, as well as an inventory of symptoms appearing after the poisoning event, with the time course of appearance of symptoms.

Amr et al. (1997) suggested that depression resulting from a high-level pesticide exposure may have unique symptoms. The population of formulators and applicators showed greater depressive neurosis (dysthymic disorder in the DSM-IV manual) with length of exposure to carbamates, pyrethroids, organophosphates and organochlorines (Amr et al. 1997). Although the study participants did not report having had acute pesticide poisonings, the investigators noted that the subjects were heavily exposed, safety measures were poor, and workers were not adequately trained in the handling of pesticides (Amr et al. 1997). The relevant point based on this study is that there were

multiple chemical exposures that used the same general detoxification pathways. OPs, carbamates, and pyrethroids utilize carboxylesterase systems. The study subjects had long-term, high-level exposures and may have had low AChE and BuChE status. The symptoms noted were similar to those seen in FSL rats and included unhappiness (anhedonia in rats), inability to cope with day to day affairs (immobility in the forced swim test), lack of energy and feelings of fatigue (anergy), insomnia, and lack of concentration.

Future epidemiological studies should examine those who have experienced a pesticide poisoning comparing those who develop depressive symptoms with those who do not. Studies should address whether a family history or personal history of affective disorder is present, whether chemical sensitivity occurs, and the specific subtypes of depression. Biological indicators of a depressed state that could be used in these studies are changes to REM sleep patterns combined with a validated set of psychiatric diagnostic tools, such as the General Health Questionnaire or the Hospital Mood Scale. Individuals should be tested for a full-range of mood disorders including anxiety, aggression and irritability, in addition to depression.

This work suggests that focusing on the details of past pesticide exposures may not be enlightening in future studies. Current exposure assessment may be adequate, particularly since it appears that it is the high-level exposures that result in long-term neuropsychiatric effects. This study suggests that future work needs to address the issues of tolerance to OPs and carbamates and the role sensitivity to these chemicals may play in the development of chronic symptoms. Mood disorders may be a marker of other neurological effects, all of which may have the same underlying biochemical mechanism.

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APPENDICES

Questions asked on the spouse questionnaire in the Agricultural Health Study and used in the spouse paper:

3. Which of the following groups *best* describes your race?
White
Black
American Indian or Alaskan Native
Asian or Pacific Islander
Other, please describe below

4. Are you of Hispanic origin? No/Yes

5. What is the highest level of schooling you have completed?
1-8 years
Some high school
High school graduate
GED (high school equivalency)
1-3 years vocational education beyond high school
Some college
College graduate
One or more years of graduate school or professional school
Something else (please describe below)

6. Altogether, how many years have you lived or worked on a farm?
Write the numbers in the box. Then fill in the matching ovals below each box.

8. *During your lifetime*, have you ever *personally* mixed or applied *any* pesticides?
(Include crop, livestock, and structural insecticides, herbicides, fungicides, and fumigants. Include pesticides used for farm use, commercial application and personal use in your home or garden.)
No Yes

a. How many years did you personally mix or apply pesticides?
1 year or less
2-5 years
6-10 years
11-20 years
21-30 years
More than 30 years

b. During those years, how many days per year did you personally mix or apply pesticides?
Less than 5 days
5-9 days
10-19 days
20-39 days
40-59 days
60-150 days
More than 150 days

c. When pesticides require mixing, what percent of the time do you personally do the mixing?
Never
Less than 50% of the time
50% or more of the time

d. What percent of the application do you personally do?
Never
Less than 50% of the time
50% or more of the time

9. In your lifetime, have you mixed or applied the following herbicides (pesticides used to kill weeds)? No=0 or Yes=1
a. Never used herbicides
b. Aatrex, Atranex or other *atrazine* products
c. Banvel, Metambane or other *dicamba* products
d. Bladex, Match or other *cyanazine* products
e. Classic or other *chlorimuron ethyl* products
f. Dual, Cycle or other *metolachlor*
g. Eradicane, Eptam or other *EPTC* products
h. Lasso, Chimiclor or other *alachlor* products
i. Lexone, Sencor or other *metribuzin* products
j. Paraquat
k. Petroleum oil/petroleum distillate
l. Prowl or other *pendimethalin* products
m. Pursuit or other *imazethpyr*
n. Roundup, Jury or other *glyphosate* products
o. Silvex or other *2,4,5 TP* products (no longer on market)
p. Sutan, Genate or other *butylate* products
q. Treflan, Trilin, Commence or other *trifluralin* products
r. 2,4-D
s. 2, 4, 5 T *(no longer on market)*
t. Other, please specify

10. *In your lifetime*, have you mixed or applied the following *crop, nursery, lawn and garden, livestock, or animal confinement area insecticides?* No=0 Yes=1
a. Never used crop or livestock insecticides
b. Ambush, Pounce, Asana, or other *permethrin or pyrethroid* products
c. Counter or other *terbufos* products
d. Dyfonate or other *fonofos* products
e. Dylox or other *trichlorfon* products
f. Forlin, Gamaphex or other *lindane* products
g. Furadan, Curaterr or other *carbofuran* products
h. Lorsban, Dursban or other *chlorpyrifos* products
i. Malathion
j. Parathion *(ethyl or methyl)*
k. Sevin, Carbamine or other *carbaryl* products
l. Spectracide, Dianon or other *diazinon* products
m. Temik or other *aldicarb* products
n. Thimet, Rampart or other *phorate* products
o. Other (please specify)

B. Crop/Livestock/Animal Confinement Area Insecticides (no longer on the market) No=0 Yes=1
a. Aldrin
b. Chlordane
c. Dieldrin
d. DDT
e. Heptachlor
f. Toxaphene
g. Other *(please specify)*

11. *In your lifetime*, have you mixed or applied the *following livestock/poultry/animal confinement area insecticides*? No=0 Yes=1
a. Never used livestock/poultry insecticides
b. Co-Ral or other *coumaphos* products
c. Ectiban, Atroban, Permetrina or other *permethrin* products
d. Vapona, Duravos or other *dichlorvos or DDVP* products
e. Other (please specify)

12. *In your lifetime*, have you injected or applied the following *fumigants* (gases or liquids that turn to gas when released; they are used in enclosed spaces or to treat soil?) No=0 Yes=1
A. Fumigants
a. Never used fumigants
b. Brom-O-Gas, Brom-O-Sol or other *methyl bromide* products
c. Phostoxin, Gastoxin or other *aluminum phoshide* products
d. Other *(Please specify)*
B. Fumigants (no longer on the market)
a. Carbon tetrachloride/ carbon disulfide (80/20 mix)
b. EDB, E-D-Bee, Bromofume or other *ethylene dibromide* products
c. Other (Please specify)

13. *In your lifetime*, have you mixed or applied the following *fungicides* (chemicals for controlling disease on crops?) No=0 Yes=1
a. Never used fungicides
b. Benlate, Tersan or other *benomyl* products
c. Bravo, Evade, Daconil 2787 or other *chlorothalonil* products
d. Orthocide, Clomitane or other *captan* products
e. Manex, Manzate, Dithane Z-78 or other *maneb or mancozeb* products
f. Ridomil, subdue or other *metalaxyl* products
g. Zirex, Corozate or other *ziram* products
h. Other (Please specify)

15. During the last growing season, did you do the following activities? Yes or No
a. Till the soil (plow, disk,cultivate)
b. Plant
c. Apply fertilizer, manure
d. Apply chemical fertilizer
e. Drive combines or other crop harvesters
f. Hand pick crops

16. *During the last growing season*, how many days per year did you work in the fields?
None
Less than 10 days
10-30 days
31-100 days
More than 100 days

17. Were you living on a farm 10 years ago? No/Yes

25. Did you *ever* have a job off a farm? No/Yes

28. For the non-farm job you held the longest, which of the following were you exposed to? *(Mark all that apply)*
Pesticides
Solvents (other than gasoline)
Gasoline
Asbestos
X-ray radiation
Grain dust
Wood dust
Cotton dust
Mineral or mining dust
Silica/sand dust
Engine exhaust
Lead solder
Welding fumes
Electroplating fumes
Lead
Mercury
Cadmium
Other metals
Pneumatic drills (vibrations)
None of these

29. How many years did you have this job?
1 year or less
2-5 years
6-10 years
11-20 years
More than 20 years

34. *During the past 12 months*, how often did you usually drink any kind of alcoholic beverage?
Never
Less than one time a month
1-3 times a month
1 time a month
2-4 times a week
Almost every day
Every day

35. *During the past 12 months*, about how many drinks would you have on a day when you drank?
Didn't drink last year
1 or 2 drinks
3 or 4 drinks
5-8 drinks
9 or more drinks

36. During your lifetime, have you smoked at least 100 cigarettes? No/Yes

37. Do you smoke cigarettes now? No/Yes

67. Who usually treats your home for these pests?
Never treat home for these pests
Myself
Someone in the household, other than myself
A professional service
Other
Don't Know

68. Who usually treats your lawn for pests (e.g., weeds, insects, or fungus)? Mark all that apply.
Lawn never regularly treated
Myself
Someone in the household, other than myself
A professional service

96. Are pesticides ever used in your vegetable garden?
No
Yes
Don't have vegetable garden

97. Are pesticides ever used on fruit in your orchard or garden?
No
Yes
Don't have orchard

104. During the past 12 months about how many times did you visit a medical doctor or medical assistant about a health concern?
None
Once
More than once

105. Has a *DOCTOR* ever told you that you had (been diagnosed with)...
A. No Yes
a. Tuberculosis
b. Melanoma of the skin
c. Other skin cancer
d. Leukemia (blood cancer)
e. Hodgkin's disease
f. Non-Hodgkin's lymphoma
g. Other cancer

h. Rheumatoid arthritis
i. Stroke
j. Myocardial infarction (heart attack)
k. Arrhythmia (irregular heart beat)
l. Angina (chest pain)
m. High blood pressure requiring medication
n. Diabetes (sugar)(other than while pregnant)
o. Asthma or reactive lung disease
p. Farmer's lung disease
q. Chronic bronchitis
r. Emphysema
s. Hay fever
t. Pneumonia (viral or bacterial)
u. Cataracts
v. Glaucoma
w. Retinal or macular degeneration
x. Detached retina
y. Goiter
z. Thyrotoxicosis/Grave's disease (excess thyroid hormone)
aa. Other thyroid disease
bb. Kidney failure requiring dialysis or transplant
cc. Chronic kidney infections or pyelonephritis
dd. Kidney stones
ee. Bright's disease, nephritis, or nephrosis
ff. Other kidney disease
gg. Shingles
hh. Eczema
ii. Mononucleosis or "mono"
jj. Scleroderma or sarcoidosis
kk. Lupus
ll. Ulcerative colitis or Crohn's disease
mm. Alzheimer's disease
nn. Parkinson's disease
oo. Amyotrophic lateral sclerosis (ALS), motor neuron disease, or Lou Gehrig's disease
pp. Epilepsy or seizures (not related to high fever)
qq. Multiple sclerosis
rr. Depression requiring medication
ss. Pesticide poisoning
tt. Solvent poisoning
uu. Lead poisoning
vv. Head injury requiring medical attention
ww. Injury from farm machinery requiring medical treatment (excluding head injury)

B. If yes, How old were you when the doctor first told you?
Younger than 20
20-39
40-59
60 or older

109. What is your sex? Female/Male

Questions asked on the private applicator enrollment questionnaire in the Agricultural Health Study and used in the farmer applicator paper:

1. What is today's date?

2. What is your birthdate?

3. Which of the following groups *best* describes your race?
White
Black
American Indian or Alaskan Native
Asian or Pacific Islander
Other, please describe below

4. Are you of Hispanic origin? No/Yes

7. What is the highest level of schooling you have completed?
1-8 years
Some high school
High school graduate
GED (high school equivalency)
1-3 years vocational education beyond high school
Some college
College graduate
One or more years of graduate school or professional school
Something else (please describe below)

10. *During your lifetime*, have you ever *personally* mixed or applied *any* pesticides?
(Include crop, livestock, and structural insecticides, herbicides, fungicides, and fumigants. Include pesticides used for farm use, commercial application and personal use in your home or garden.)
No/Yes

c. How many years did you personally mix or apply pesticides?
1 year or less
2-5 years
6-10 years
11-20 years
21-30 years
More than 30 years

d. During those years, how many days per year did you personally mix or apply pesticides?
Less than 5 days
5-9 days
10-19 days
20-39 days
40-59 days
60-150 days
More than 150 days

c. When pesticides require mixing, what percent of the time do you personally do the mixing?
Never
Less than 50% of the time
50% or more of the time

j. What percent of the application do you personally do?
Never
Less than 50% of the time
50% or more of the time

14, How often, if every, have you had the following symptoms that you think may be related to your using pesticides?
a. been excessively tired?
b. had headaches/dizziness?
c. had nausea or vomiting?
d. had skin irritation?
e. had eye irritation?
f. had chest discomfort?
g. felt nervous or depressed?

Answer choices: Never or rarely, sometimes, frequently/almost always

15. As a result of using pesticides, how often have you:

a. seen a doctor?
Answer choices: Never, once, twice, 3 or more times

17. What type of protective equipment do you generally wear when you personally handle pesticides? (Mark all that apply)
Do not personally handle pesticides

21. *During the past 12 months*, how often did you usually drink any kind of alcoholic beverage?
Never
Less than one time a month
1-3 times a month
1 time a month
2-4 times a week
Almost every day
Every day

23. During your lifetime, have you smoked at least 100 cigarettes? No/Yes

38. Do you smoke cigarettes now? No/Yes

31. What are the major income producing crops and animals you are currently raising on a farm?
Don't work on a farm
No crops or animals
Beef cattle
Dairy cattle
Hogs/swine
Poultry

Sheep
Eggs
Other farm animals
Apples
Alfalfa
Blueberries
Cabbage
Christmas trees
Corn, popcorn, field corn seed corn, sweet corn
Cotton
Cucumbers
Grapes
Green peppers
Hay
Oats
Peaches
Peanuts
Potatoes
Snapbeans
Sorghum
Soybeans
Strawberries
Tomatoes
Tobacco
Watermelon
Wheat
Other fruit
Other vegetables
Other small grains

32. Last year, how many acres were planted on the farm(s) where you worked (whether or not you owned the farm)?
Didn't work on a farm
None
Less than 5 acres
5-49 acres
50-199 acres
200-499 acres
500-999 acres
More than 1,000 acres

33. Last year, how many poultry were there on the farm(s)? (Report the most poultry you had last year at any one time.)
Didn't work on a farm
None
Less than 50
50-99
100-499
500-999
1,000-10,000
More than 10,000

34. Last year, how many livestock (other than poultry) were there on the farm(s)? (report the most livestock you had last year at any one time.)
Didn't work on a farm
None
Less than 50
50-99
100-499
500-999
1,000 or more

37. What is your sex? Male/Female

38. What is your current marital status?
Married or living as married
Divorced or separated?
Widowed
Never married

Take-home questionnaire:
2. Over your lifetime, how many years have you lived or worked on a farm?
Less than 5 years
5-10 years
11-20 years
21-30 years
Over 30 years

25. Have you ever had an incident or experience while using any type of pesticide which caused you unusually high personal exposure? Yes/No

51. Did you ever have a job off a farm? Yes/No

86. During the past 12 months about how many times did you visit a medical doctor or medical assistant about a health concern?
None
Once
More than once

87. Has a *DOCTOR* ever told you that you had (been diagnosed with)...
A. No Yes
a. Rheumatoid arthritis
b. Stroke
c. Myocardial infarction
d. Arrhythmia
e. Angina
f. High blood pressure requiring medication
g. Diabetes (sugar) (other than while pregnant)
h. Asthma or reactive lung disease
i. Farmer's lung disease
j. Chronic bronchitis
k. Emphysema

l. Hay fever
m. Pneumonia (viral or bacterial)
n. Cataracts
o. Glaucoma
p. Detached retina
q. Retinal or macular degeneration
r. Goiter
s. Thyrotoxicosis/Grave's disease (excess thyroid hormone)
t. Other thyroid disease
u. Kidney failure requiring dialysis or transplant
v. Chronic kidney infections or pyelonephritis
w. Kidney stones
x. Bright's disease, nephritis, or nephrosis
y. Other kidney disease
z. Shingles
aa.Eczema
bb Mono
cc Scleroderma or sarcoidosis
dd Lupus
ee Ulcerative colitis or Crohn's disease
ff Parkinson's disease
gg Amyotrophic lateral sclerosis
hh Epilepsy or seizures (not related to high fever)
ii. Multiple sclerosis
jj Depression requiring medication or shock therapy
kk Pesticide poisoning
ll Solvent poisoning
mm Lead poisoning
nn Head injury requiring medical attention
oo Injury from farm machinery require medical treatment (excluding head injury)

C. If yes, How old were you when the doctor first told you?
Younger than 20
20-39
40-59
60 or older

90. Approximately how often during the last 12 months have you experienced the following?
v. Feeling depressed, indifferent, or withdrawn
Possible responses: Never, one a year, once a month, once a week, more than once a week