PESTICIDE EXPOSURE AND END-STAGE RENAL DISEASE AMONG PESTICIDE APPLICATORS AND THEIR SPOUSES IN THE AGRICULTURAL HEALTH STUDY

Jill Farnell Lebov

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Approved by:

Lawrence S. Engel

David Richardson

Jane Hoppin

Dale Sandler

Susan Hogan

ABSTRACT

Jill Lebov: Pesticide Exposure and End-Stage Renal Disease among Pesticide Applicators and their Spouses in the Agricultural Health Study
Under the direction of Lawrence S. Engel

Experimental studies suggest a relationship between pesticide exposure and renal impairment, but epidemiological research on the long-term effects of chronic low-level and acute pesticide exposure on renal disease risk is limited.

This study investigated the relationship between end-stage renal disease (ESRD) risk and 1) long-term use of and exposure to specific pesticides; 2) short-term high-level pesticide exposures; and 3) farming and household factors that may increase exposure to pesticides, among male licensed pesticide applicators (N=55,580) and their wives (N=32,099) in the Agricultural Health Study (AHS).

AHS participants reported pesticide use and exposure via self-administered questionnaires at enrollment (1993-1997). Associations between ESRD and pesticide exposures were estimated with Cox proportional hazard regression models controlling for age at enrollment, state of enrollment (applicator analyses only), and personal use of any pesticide (wives analyses only). ESRD cases were identified via linkage to the United States Renal Data System (USRDS). Standardized incidence ratios (SIRs) were calculated to compare ESRD incidence rates in the AHS cohort to the general population.

We identified 320 and 103 ESRD cases diagnosed between enrollment and 31 December 2011 among pesticide applicators and wives, respectively. Among applicators, ESRD risk was elevated with use of the fungicide metalaxyl, and the herbicides imazethapyr, paraquat, and petroleum oil, with positive exposure-response trends observed for paraquat, pendimethalin, and the insecticide chlordane. Medical visits due to pesticide use were associated with ESRD.

Among wives who never applied pesticides, ESRD risk was significantly associated with husbands' ever use of paraquat and butylate, with a positive exposure-response trend observed for husband's cumulative use of these pesticides. Positive associations were observed with private well proximity to pesticide mixing areas, washing pesticide-exposed clothing with the family wash, and spending >10 hours in the sun during the growing season, though estimates were imprecise. ESRD incidence rates were lower among applicators and wives compared to the general population.

Our findings support a possible association between ESRD risk and chronic exposure (both direct and indirect) to certain pesticides and suggest that pesticide exposures resulting in medical visits may increase the risk of incident ESRD.

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LIST OF ABBREVIATIONS

2,4,5-T	2,4,5-Trichlorophenoxyacetic acid
2,4,5-TP	2,4,5-Trichlorophenoxyacetic Propanoic acid
2,4-D	2,4-Dichlorophenoxyacetic acid
AHS	Agricultural Health Study
AIC	Akaike Information Criteria
ARIC	Atherosclerosis Risk In Communities
BMI	Body Mass Index
BRFSS	Behavioral Risk Factor Surveillance System
CI	Confidence Interval
CKD	Chronic Kidney Disease
CMS	Centers for Medicare and Medicaid Services
DAG	Directed Acyclic Graph
DB	Diabetes
DDT	Dichlorodiphenyltrichloroethane
eGFR	Estimated Glomerular Filtration Rate
ESRD	End-Stage Renal Disease
FSD	First ESRD service date
HPEE	High pesticide exposure experience
HR	Hazard Ratio
IA	Iowa
MSAS	Minimally sufficient adjustment set
NC	North Carolina
NHANES	National Health and Nutrition Examination Survey
NHIS	National Health Information Survey

NSAIDS	Non-steroidal anti-inflammatory drugs
OC	Organochlorine insecticides
OP	Organophosphate insecticides
RENDER	Renal Data Extraction and Referencing
ROS	Reactive Oxygen Species
RR	Risk Ratio
SIR	Standardized Incidence Ratio
TH	Take-home questionnaire
UNOS	United Network for Organ Sharing
USRDS	United States Renal Data System

CHAPTER 1: INTRODUCTION AND BACKGROUND

Introduction

Pesticide exposure has been linked to a variety of adverse chronic health outcomes, including diabetes mellitus (1-3), gestational diabetes (4), obesity (5), and cancer (6, 7). While pesticides are associated with diseases that may contribute to end-stage renal disease (ESRD), little is known about the potential association between pesticide use and kidney disease. A broad range of pesticides, including organophosphates (8), organochlorines (9), carbamates (10), pyrethroids (11) and triazine herbicides (12) have been shown to cause renal damage and dysfunction in animal toxicity studies. Case reports of both fatal and non-fatal pesticide poisoning have described nephrotoxic effects of insecticides, herbicides, fungicides, and fumigants (13-17). Yet, the impact of long-term pesticide exposure on the human kidney remains unknown. Studies conducted in El Salvador, Nicaragua, and Sri Lanka indicate an elevated prevalence of chronic kidney disease among agricultural workers (18-21); pesticide exposure is postulated to be a contributor to kidney disease in these regions, but existing evidence has not confirmed this hypothesis (18-20). The only study to assess agrochemical exposure and ESRD found frequent exposure to insect or plant spray to be associated with increased ESRD risk (22). These studies lack specificity with regard to chemical type and have not been able to adequately assess the long-term effects of chronic or acute pesticide exposure on ESRD risk.

A goal of this dissertation was to examine associations between chronic use of specific pesticides and end-stage renal disease (ESRD) among a large cohort of pesticide applicators and their spouses in Iowa and North Carolina. We sought to evaluate the impact of ever use of specific pesticides among applicators and spouses, as well as the relationship between cumulative use of specific pesticides and ESRD risk among applicators. Further, we assessed the relationship between short-term high level pesticide exposure and ESRD risk among pesticide applicators in this cohort. A number of studies have

documented and quantified residential pesticide exposure from carry-home contamination by agricultural workers and spray drift from nearby fields (23-25), but research is lacking on the impact of these exposure opportunities on renal disease risk. A second aim of this dissertation was to evaluate the role of husbands' pesticide use and household and farming factors associated with pesticide exposure in ESRD risk among farm wives, an understudied population.

Analyses conducted within a cohort of pesticide applicators and their spouses facilitated understanding of the relationship between use of specific pesticides and ESRD. It is also important to compare the incidence of ESRD among an occupational cohort with relatively high lifetime pesticide exposure to disease incidence in the general population. Because occupational cohorts are often healthier than the general population, comparisons of disease incidence between these two populations are likely to be biased. Methods to account for differential risk factor distributions are necessary to address this healthy worker effect. Thus, an additional objective of this dissertation was to compare incidence rates of ESRD in the AHS population to rates in the general population using standardized incidence ratios, adjusted for the strongest known ESRD risk factor, diabetes mellitus.

The Agricultural Health Study (AHS), a large prospective cohort study of pesticide applicators and their spouses in North Carolina and Iowa, was established to assess the role of pesticide use and other environmental factors in relation to morbidity and mortality among pesticide applicators and their families (26). The United States Renal Data System (USRDS) maintains a database with diagnostic and demographic information on all people treated for ESRD since 1995. AHS data linked with USRDS data were be used to address the following aims:

SPECIFIC AIMS

Aim 1: Evaluate the relationship between pesticide use, short-term high level pesticide exposure, and ESRD among pesticide applicators.

Sub-aims:

A. Determine the magnitude of association between ESRD risk and 1) ever use and 2) cumulative pesticide use and incident ESRD for specific pesticides.

Hypothesis: Risk of ESRD increases with increasing cumulative pesticide use in an exposure-response manner, and is associated with use of pesticides that have a clear nephrotoxic effect in humans as described in the literature (e.g. paraquat).

B. Determine the magnitude of association between short-term high level pesticide exposure events and incident ESRD among pesticide applicators.

Hypotheses:

i. *Risk of ESRD is increased among those who report visiting a medical professional due to pesticide use compared to those who do not.*
ii. *Risk of ESRD is increased among those who report a diagnosis of pesticide poisoning compared to those who do not.*
iii. *Risk of ESRD is increased among those who report an incident of unusually high personal exposure to pesticides compared to those who do not.*

Aim 2: Evaluate the relationship between pesticide use, indirect pesticide exposures, and ESRD among spouses of pesticide applicators. Note: we restricted these analyses to female spouses; therefore, spouses will be referred to as wives throughout the remainder of this chapter.

Sub-aims:

A. Determine the magnitude of association between ever use of pesticides (specific and chemical class) and incident ESRD

Hypothesis: This is an exploratory analysis that facilitates comparison of pesticide risk patterns between the spouse and applicator sub-cohorts. The literature indicates that renal damage is a common result of high level exposure to several individual pesticides. We expect that certain pesticides with known nephrotoxic effects (e.g. paraquat) will have stronger associations with ESRD.

B. Determine the magnitude of association between ESRD risk and indirect exposure to the applicator husbands' ever and cumulative use of specific chemicals, among wives who do not apply pesticides themselves.

Hypotheses:

i. *The risk of ESRD increases with increasing cumulative use of specific pesticides by the husbands, in an exposure-response manner. The scientific literature does provide some evidence that direct exposure to certain pesticides may lead to renal damage, but research is lacking on the renal effects of indirect or 'bystander' exposure to specific chemicals. We hypothesize that the pesticides associated with ESRD in Aim 1 may be associated with ESRD among spouses in Aim 2.*

C. Determine the magnitude of association between ESRD risk and other pesticide exposure routes, such as those incurred through household hygiene practices and farming activities, among all wives

i. *Risk of ESRD is increased among wives indirectly exposed to pesticides through household hygiene practices that increase residential pesticide contamination, those who live in close proximity of the home to area where pesticides are mixed and applied, and those who worked more days in the field in the last growing season*

Aim 3: Compare the incidence of ESRD in the Agricultural Health Study cohort to that of the general populations of Iowa and North Carolina

Hypothesis: After adjustment for important demographic factors and for diabetes, estimated ESRD incidence in the AHS will be higher than that of the general population, in Iowa and North Carolina.

Background

In 2010, more than half a million Americans were receiving treatment for ESRD. Mortality among people with ESRD is ten times greater than among Medicare patients of similar age (27). Though ESRD cases make up only 1.3% of Medicare patients, ESRD care accounts for 7.5% of Medicare spending, amounting to $47.5 billion per year. Whereas clinical pathology of ESRD is relatively well-understood and commonly studied, little is known about the impact of environmental and occupational factors on risk of ESRD. Laboratory studies and case reports suggest that pesticides are nephrotoxic at high doses, but the long-term effects of chronic low-level and acute non-fatal pesticide exposures on human renal outcomes have not been studied.

Pesticide use and associated disease

Widespread use of pesticides in the United States results in frequent human exposure; biological monitoring studies have shown that low-level exposures to pesticides and pesticide residues are ubiquitous in the adult general population in the United States (28). Farmers and farmworkers are exposed to pesticides through mixing, loading and applying pesticides, or while performing duties related to harvesting and planting crops (29). Pesticides were developed to increase crop production by exterminating or repelling insects, weeds, and fungi; however, pesticides have been found to adversely affect many non-target species, including humans. Pesticide exposure has been linked to a variety of adverse chronic health outcomes among agricultural workers, including diabetes mellitus (1-3), gestational diabetes (4), cancer (7), pregnancy-induced hypertension (30), respiratory diseases (31, 32), and certain neurologic outcomes (33, 34).

The general population is exposed to pesticides and their metabolites in several ways. People living in areas near regular pesticide application may experience pesticide exposure through drift during pesticide application and through ingestion of public drinking water sources contaminated by pesticide runoff. Proximity of household to pesticide application area is positively correlated with levels of pesticides found in household dust (23, 35). And, several large drinking water surveys have found

widespread contamination of community water systems and domestic wells by pesticides and pesticide degradates (36-39). Chlorophenoxy herbicides, and carbamate, pyrethroid, and organophosphate insecticides are common in home and lawn care (40), several of which are considered to be moderately to highly toxic depending on the formulation (41). Diet may also be an important exposure route for the general population (42). Farm families' exposures are higher and more varied than the general population, and may include 'take-home' exposures, by which pesticide residues are tracked into the home on work boots and clothing. Observed levels of urinary pesticide metabolites were higher among young children of pesticide applicators compared to children in non-agricultural families (23, 24), and among families living in farming households vs. non-farming households (43). Though the United States E.P.A. has developed criteria for the allowable levels for many pesticides in foods, drinking water, and environmental resources, the human health effects of prolonged low-dose exposure to pesticides are unknown for many of the pesticides currently on the market.

Pesticide poisoning is also a significant public health concern. Though the actual number of annual pesticide poisoning incidents in the United States is unknown, approximately 90,000 pesticide exposures were reported to the American Association of Poison Control Centers in 2010 (44). An estimated 10,000-20,000 physician-diagnosed pesticide poisonings are reported to occur each year among U.S. agricultural workers (45). This number is thought to represent a considerable underestimate of the actual number of poisonings, due to lack of access to medical care for some farmworkers and potential misdiagnosis by clinicians. Pesticide poisoning can lead to damage to various organ systems and death (46), and the renal system is affected by poisoning with a wide range of pesticides (46). Despite the known health hazards of pesticide poisoning, little is known about the relationship between short-term high-level or chronic low-level pesticide exposure and renal disease at the population level.

Kidney disease – definition, risk factors and outcomes

Kidney disease is characterized by kidney dysfunction, kidney damage, or both. Kidney dysfunction is defined based on the excretory capacity of the kidney. Healthy kidneys filter blood for waste products, which are excreted in the urine. Thus, kidney dysfunction is assessed through measurement of metabolic by-products, including creatinine, in blood and urine. Serum creatinine is used to estimate glomerular filtration rate (eGFR), which is the best indicator of overall renal function (47). Kidney damage is defined by structural abnormalities or functional abnormalities other than decreased GFR. Kidney damage is evidenced by one or all of the following: increased glomerular permeability measured by urine albumin to creatinine ratio; abnormalities in urinary sediment such as the presence of red or white blood cell casts, oval fat bodies, or renal tubular epithelial cells; imaging abnormalities, such as renal cysts, small or enlarged kidneys, scarring, etc.; and evidence of renal tubular syndromes, such as renal tubular acidosis and nephrogenic diabetes insipidus (48).

Chronic kidney disease (CKD) is defined as an eGFR<60 mL/min per 1.73 m^2 and/or evidence of kidney damage for 3 or more months (49). 'Per 1.73 m^2' refers to body surface area, and 1.73 m^2 is the normal mean body surface area value for young adults (50). Kidney function declines naturally with age, but CKD is characterized by more rapid decline. CKD progresses to chronic renal failure over a period of months or years, depending on the severity of disease and comorbid conditions. Major pathological risk factors for CKD include diabetes, hypertension, obesity, cardiovascular disease, and congenital renal abnormalities. Demographic risk factors include older age and African American race. Lifestyle risk factors are less clear – alcohol use does not appear to be associated with CKD risk (51), whereas heavy NSAID use and heavy smoking do appear to be associated with CKD risk (52, 53). The most common types of CKD are diabetic nephropathy, hypertensive nephrosclerosis, and glomerulonephritis (27, 48). A less common, but toxicologically important, type of chronic kidney disease is tubulointerstitial disease, which results from drug and toxin-induced chronic tubulointerstitial nephritis (54). Prolonged exposure to

nephrotoxic agents may eventually lead to permanent changes in the tubulointerstitium, such as tubular atrophy and interstitial fibrosis (55).

The final stage of chronic kidney disease is kidney failure, defined as either 1) eGFR less than 15 mL/min per 1.73 m^2, which is accompanied in most cases by signs and symptoms of uremia, or 2) a need to start renal replacement therapy (dialysis or transplantation) (49). Kidney failure treated by renal replacement therapy is known as end stage renal disease (ESRD), which is the operational definition used to define the population base in the USRDS. In 2010, there were 116,946 incident cases of ESRD in the U.S., with an age-, gender-, and race-adjusted annual rate of 350 cases per million population. In that same year, there were approximately 91,000 ESRD deaths and 600,000 prevalent cases. ESRD is characterized by high rates of mortality and morbidity. Complications of reduced GFR include an increased risk of acute kidney injury, infection, cognitive impairment, impaired physical function, and cardiovascular disease (48). The cardiovascular death rate in the dialysis population is almost 40 times greater than in the general population (56).Only 51 percent of dialysis patients, and 82 percent of those who receive a preemptive transplant, are still alive three years after the start of ESRD therapy (27).

ESRD incidence rates increase with age and are higher among males vs. females and among people living in the southeastern United States compared to other U.S. regions (27). Black or African Americans are at highest risk, with an incidence rate almost three times that of the national average (27). The prevalence of early stages of chronic kidney disease is approximately 50 times greater than the prevalence of kidney failure (57). The leading causes of ESRD are diabetes and hypertension, with approximately 44% of ESRD cases attributable to diabetes and 28% attributable to hypertension (27). Hsu et al (2009) conducted a large prospective cohort study of Kaiser Permanente members and found the following additional factors measured at baseline independently increased the hazard of ESRD over an average follow-up period of 25 years: obesity, high serum uric acid level, high proteinuria, and elevated serum creatinine (22). The latter three conditions would be expected to predict ESRD, as they often occur

in CKD. Additionally, the use of non-steroidal anti-inflammatory drugs and acetaminophen (52, 58-61) has been associated with CKD and ESRD risk.

The literature on occupational risk factors for ESRD is limited. Hsu et al (2009) identified significant univariate associations with ESRD and various broad categories of occupational exposures, including: lead or other metal fumes; asbestos, cement, or grain dust; ammonia, chlorine, ozone, or nitrous gas; chemicals, cleaning fluids, or solvents; engine exhaust fumes; extreme heat; and silica, sandblasting, grinding, or rock dust. In case-control studies of ESRD, the association with fumes and solvents has been inconsistent (62-65), although different definitions of the exposures and the outcome make it difficult to compare the results of these studies. However, cohort studies do suggest solvent exposure as a potential risk factor for the development of ESRD (66, 67) and more rapid progression to ESRD among those with CKD (68). ESRD has consistently been associated with occupational exposure to silica (63, 69, 70).

Evidence for a relationship between pesticide exposure and ESRD

The anatomic, physiologic, and biochemical features of the kidney predispose it to adverse effects of diverse environmental chemicals. Despite their relatively small size, kidneys receive twenty to twenty-five percent of cardiac output. This large blood flow results in high concentrations of toxicant delivery to the kidney. The kidneys are the primary organ for excretion of xenobiotics, and renal enzymes can concentrate and metabolize xenobiotic compounds in the kidney. The urine concentrating ability of the kidney may further increase concentrations of toxicants localized to the kidney, potentially leading to obstruction of tubular flow and damage to nephron and tubular cells (71).

Pesticide poisoning case studies, animal models, and *in vitro* laboratory research provide evidence for a damaging effect of both acute and chronic pesticide exposure on renal function. The most commonly reported pesticides implicated in human renal damage are organophosphates, though other pesticides are known to be nephrotoxic at high levels. Acute kidney injury (AKI), an abrupt (within 48

hours) reduction in kidney function (also called acute renal failure)(72), is a frequently observed outcome of pesticide poisoning. Acute kidney injury has been reported as a result of intoxication by the organophosphates (OPs) dimethoate (13) and malathion (73), and by the herbicide paraquat (14, 17, 74). Poisoning by aluminum phosphide (fumigant), maneb (fungicide), and glyphosate and bentazone (herbicides) also resulted in acute renal failure in several patients (15, 16, 75-77). In a study of 52 patients with endosulfan (organochlorine) poisoning, Moon and Chun (2009) found that 27% experienced acute kidney injury (78). Commonly observed renal symptoms in pesticide poisoning include acute tubular necrosis, hematuria, and proteinuria (46).

Acute tubular necrosis has also been seen in animal studies of pesticide nephrotoxicity, along with other evidence of renal damage from pesticide exposure. Nephrotoxicity of organochlorines (OCs) has been particularly well-studied in animal models. Incidence of glomerular lesions was significantly higher among foxes exposed to OC pesticides in food over a period of 6 months compared to unexposed foxes (79). Relatedly, time to onset of renal impairment was significantly decreased among rats treated with OC pesticides (chlordecone, methoxychlor, and o,p´-dichlorodiphenyltrichloroethane (o,p´- DDT)) compared to controls (9). The effect of chronic endosulfan exposure in rats was reviewed by Naqvi et al (1993), who found the chemical to be nephrotoxic, indicated by degeneration in the proximal convoluted tubules and necrosis of the tubular epithelium (80). The destructive properties of endosulfan on the kidney were confirmed by Choudhary et al (2002) who also observed evidence of deteriorating kidney function with longer exposures (81).

Other pesticides have been implicated in renal damage and dysfunction. Chargui et al (2012) observed lesions within kidney tubules and severe alterations of the glomeruli among rats exposed to low doses of deltamethrin (synthetic pyrethroid insecticide) over time (11). Treatment with organophosphate insecticides caused a dose- and time-dependent renal tubular cytotoxicity as well as evidence of kidney dysfunction in rats (8, 82, 83). This effect was also observed *in vitro (8).* Rats exposed to carbofuran (carbamate insecticide) exhibited significantly poorer renal function after 28 days compared to control

rats (10). Kackar et al (1999) observed morphological changes in the renal tubules of rats exposed to Mancozeb (dithiocarbamate fungicide), including tubular necrosis with alterations in tubular epithelial lining (84). Oulmi et al (1995) observed atrazine (triazine herbicide) dose-dependent degenerative cytopathology in the renal tubules of rainbow trout (12). Uyanikgil et al (2009) found that sub-acute 2,4-D administration induces dose-dependent histopathological deleterious effects in the rat kidney cortex (85).

Evidence suggests that pesticide exposure may harm the kidneys through oxidative stress and resulting cell damage. Exposure to a wide variety of pesticides is associated with the generation of reactive oxygen species (ROS) and altered activity of antioxidant enzymes in animal models and humans (86). *In vitro* and in rats, increased antioxidant enzyme activity has been observed in relation to pyrethroid (87) and organophosphate (88) exposure, and is thought to represent an adaptive response which initiates enzyme activity to scavenge free radicals. In contrast, analyses of organophosphate insecticide exposure among agricultural workers (89, 90) and rats (82) and maleic hydrazide herbicide exposure *in vitro* (91) have observed a decrease in antioxidant enzymes activity. The decrease in these enzymes may indicate an inhibition of antioxidant enzymes resulting from binding of oxidative molecules produced during pesticide metabolism (89). Whether enzymatic activity increases or decreases likely has to do with dose. Small doses of pesticides generate ROS which induce enzyme activity to balance the redox system. Large doses generate a quantity of ROS that may overwhelm the capacity of cellular antioxidant enzymes (92). Changes in these enzymes, regardless of direction, are indicative of oxidative stress, and biomarkers of oxidative stress are increased among patients with chronic renal failure (93).

The generation of free radicals may also induce lipid peroxidation, which occurs when free radicals pull electrons away from lipids in cell membranes (86), thereby causing deterioration of cell membranes and eventual apoptosis. Cell damage caused by lipid peroxidation is reflected in part by glomerular lesions and renal tubular necrosis observed in *in vivo* and *in vitro* pesticide exposure studies (8, 10, 71).

Other possible pathways for pesticide-induced renal damage may involve DNA degradation and cell-mediated immune reaction, though these pathways are not well-characterized with regard to renal function in the scientific literature. DNA damage observed with exposure to organophosphate and organochlorine insecticides (94-96) may contribute to cell degradation; however, this result may simply be an effect of oxidative stress caused by these pesticides rather than a separate pathway. Immune system response to xenobiotics can induce inflammation in the kidney, resulting in eventual tubular injury (55). Pesticides have been shown to affect the immune system (97), but the effect of pesticide-specific immune response on kidney function is poorly understood.

Evidence of renal damage and dysfunction, oxidative stress, and DNA damage due to pesticide exposure is summarized in Table 1.1. The list of pesticides presented in Table 1.1 is by no means exhaustive, but this list is representative of the literature on kidney pathology associated with pesticide exposure in animals and humans. In the table, the codes A and H indicate that evidence of an effect has been observed in animals and humans, respectively. Animal studies were conducted in rats, unless otherwise noted. Empty cells do not imply that no evidence exists; rather, empty cells indicate that evidence of an effect has not been described in the studies included in this literature review. For more detail on findings from these studies, see Appendix 3.

Table 1.1: Evidence of change in kidney pathology due to pesticide exposure in human and animal studies

Chemical class and specific pesticide	Evidence of damage to kidney tissues or kidney cells*	Evidence of impairment of renal function	Evidence of oxidative stress	Evidence of DNA Damage
Organophosphate insecticide				
Diazinon	A	A	A	
Malathion	A	H		
Dimethoate	H, A (fish)			
Chlorpyrifos, methyl parathion, and malathion			A	
Pyrethroid insecticide				
Deltamethrin	A		A	A
Organochlorine insecticide				
Endosulfan	A	A	A(fish)	A(fish)
Chlordecone, methoxychlor, and o,p'-DDT	A	A		
Organochlorines (multiple)**	A (fox)			
Carbamate insecticide				
Carbofuran		A	A	
Carbaryl		A	A	
Dithiocarbamate fungicide				
Maneb/Mancozeb	A	H		
Triazine herbicide				
Atrazine	A (fish)			
Chlorophenoxy herbicide				
2,4,D	A			
Phosphonate herbicide				
Glyphosate		H, A (observed change in rats not indicative of renal dysfunction)	A (mixed)	
Other herbicide				
Bentazone		H		
Paraquat		H		
Fumigant				
Aluminum phosphide		H		

*Damage to tissues and cells includes: degeneration of tubular epithelial cells and glomerular capsules, necrosis of proximal tubules, glomerular, tubular, and interstitial lesions, glomerulosclerosis, degenerative effects in kidney cortex, proliferative glomerulonephritis

** Sonne et al indicated that the food they fed foxes contained all of the following pesticides and pesticide metabolites: 1,3-DCB, 1,4-DCB, 1,2-DCB, 1,3,5-TCB, 1,2,4-TCB, 1,2, 3-TCB, Hexachlorobutadiene, 1,2,3,4-TTCB, PECB, a-HCH, HCB, Pentachloroanisole, b-HCH, g-HCH(Lindane), Heptchlor, Aldrin, Octachlorostyrene, Heptachlor epoxide, Oxychlordane, g-Chlordane, a-Endosulfan, o,p-DDE, a-Chlordane, trans-Nonachlor, Dieldrin, p,p-DDE, o,p-DDD, Endrin, b-Endosulfan, cis-Nonachlor, p,p-DDD, o,p-DDT, p,p-DDT, Methoxychlor, Mirex.

H=Evidence observed in human study; A= Evidence observed in animal study

Importantly, tubulointerstitial and glomerular cell damage, such as that observed in relation to pesticide exposure, can initiate a feed-forward loop of kidney injury and progressive loss of function that leads to ESRD. This occurs through two hypothesized cyclic models of CKD progression. The "overload

hypothesis" suggests that initial kidney injury results in a decreased number of functioning nephrons. In response, remaining nephrons compensate to maintain kidney function, resulting in further nephron damage and loss. In the "fibrosis hypothesis," kidney insults cause tubulointerstitial damage, resulting in inflammation and subsequent damage to the tubulointerstitium (55). Clinically, acute kidney injury (AKI) is a frequent outcome of pesticide poisoning among humans (46), and is associated with subsequent renal disease. Those who have experienced an AKI event are generally believed to be at significantly higher risk of developing chronic kidney disease and ESRD (98-100). Thus, it is possible that acute and/or chronic pesticide exposures could increase the risk of ESRD through one or more kidney insults.

Other xenobiotic chemicals are thought to induce oxidative stress and cause tubular and interstitial damage. Altered antioxidant defense has been seen with solvent exposure (101), a potential risk factor for kidney disease. Additionally, the pattern of increased lipid peroxidation with decreased antioxidant enzyme activity is observed in exposure to cadmium and mercury (102, 103), which are known nephrotoxins (103, 104). The pathogenesis of analgesic abuse nephropathy is thought to involve oxidative stress, resulting in tubular atrophy, interstitial fibrosis, and inflammation (54). Lead nephropathy is characterized by interstitial nephritis and fibrosis and tubular atrophy (105, 106). With initial high-level lead poisoning, the proximal tubules are injured, and continued exposure results in a chronic interstitial nephritis. Prolonged ingestion of aristolochic acid, a plant alkaloid product of the Chinese herb *Aristolochia fangchi*, induces nephropathy characterized by severe tubulointerstitial fibrosis with minimal glomerular injury (71, 106). Results from epidemiological research among the agricultural populations in Sri Lanka and Central America also suggest interstitial rather than glomerular etiology of kidney disease (18, 20, 107, 108). Because the former type of disease is primarily induced by exposure to pharmaceuticals and environmental chemicals, and pesticides are known to be nephrotoxic, the hypothesis of pesticide exposure as a significant risk factor for renal disease among these agricultural populations is plausible.

Existing epidemiological research on the potential association between pesticide exposure and kidney disease is minimal, and no longitudinal research on this relationship has not evaluated specific chemicals. In Nicaragua, El Salvador, and Sri Lanka, prevalence of chronic kidney disease (CKD) is elevated among agricultural workers compared to those who have never worked in agriculture (18, 19, 109, 110). Prevalence rates are particularly high for fieldworkers compared to non-fieldworkers (adjusted odds ratio (OR): 2.48; 95% CI:1.59,3.89) (19) and increase with increasing duration of agricultural work (Lebov et al 2014, under review), independent of age, sex, diabetes, and hypertension. This observation has led investigators to postulate pesticide exposure as a main contributor to kidney disease in these regions, though this link remains to be confirmed. A significant crude association was found between pesticide use and CKD in the Sri Lankan study (OR: 2.24; 95% CI: 1.45, 3.45) (110). Similarly, studies by O'Donnell et al (2011) and Sanoff et al (2010) found significant univariate associations between pesticide exposure and CKD, but these associations were no longer significant after adjustment for age, sex, and CKD risk factors (O'Donnell: Adjusted OR:1.32; 95% CI: 0.66, 2.64; Sanoff: Adjusted OR: 1.38; 95% CI: 0.90,2.11) (19, 20). Payán-Rentería et al (2012) found no difference in urine albumin or creatinine levels among male farmers in Mexico who applied pesticides in the previous season compared to those who did not apply in the previous season. However, the study was very small (25 exposed; 21 unexposed), and the similar occupational histories of the two groups likely diminished any effect of exposure that would be seen. Though these studies were not designed to evaluate the effect of chronic pesticide exposure on kidney disease incidence, this body of research does suggest a relationship that deserves further exploration.

Epidemiological analyses of the influence of pesticides on biological markers of kidney function have been inconclusive. The only study to evaluate specific agrochemicals and biological measures of renal function was conducted among non-agricultural populations in India and found higher blood levels of organochlorines among CKD cases (eGFR $\leq$60 mL/min/1.73 m^2), compared to controls (111). The authors hypothesized that filtration deficiency inherent among CKD patients may allow for accumulation

of organochlorines in blood. Hernandez et al (2006) evaluated the cross-sectional relationship of pesticide exposure and serum creatinine levels at two time points among of a cohort of 106 intensive agriculture workers. Investigators observed a low ($r = -0.24$), but significant, inverse correlation between cumulative pesticide exposure and serum creatinine in multiple linear regression models. Serum creatinine levels increase with worsening kidney function; therefore the observed inverse relationship seems to suggest that pesticide exposure was associated with better kidney function (112). However, it is possible that those who have higher serum creatinine values (i.e. less healthy individuals) work less and therefore use pesticides less heavily.

The only study to assess the relationship between ESRD risk and agrochemical exposures observed an increased risk of ESRD among those reporting to have worked in a place with "frequent or daily exposure to insect or plant spray" (Unadjusted hazard ratio: 1.78; 95% CI: 1.36-2.34) (22). The findings of this study support the hypothesis of pesticide exposure as a risk factor for ESRD; however, the study design did not permit assessment of exposure-response relationships, impact of specific pesticides, or varying intensity or chronicity of exposure. Additional studies with considerably more detailed exposure and covariate information are required to adequately assess pesticide exposure as an independent risk factor for ESRD.

Innovation and Significance

Though there have been several studies assessing pesticide exposure and CKD, these analyses have all been cross-sectional or case-control studies, and have either focused on a single pesticide or pesticide class, or broadly assessed ever/never exposure to any pesticide. Furthermore, these studies have been characterized by small sample sizes, bias in case and control ascertainment, and potential misclassification of the outcome. The only study to attempt to evaluate pesticide exposure as a risk factor for incident ESRD addressed occupational, but not necessarily agricultural, exposure and specific chemicals were not evaluated. Previous studies have not been able to characterize the long-term impacts of chronic exposure to specific pesticides or acute non-fatal pesticide exposures on the human renal

system, nor have they evaluated factors that may affect degree of exposure, such as pesticide application methods or use of personal protective equipment.

The present study improves upon prior research in several ways. First, we were able to evaluate associations of ESRD risk with a wide range of specific chemicals which vary in toxicity and frequency of use. The AHS data include information about lifetime use of 50 specific chemicals, which can be categorized and reviewed for exposure-response trends. Also, we were able to adjust for pesticide application methods, repair of pesticide application equipment, and use of personal protective equipment by analyzing intensity-weighted exposure variables for each pesticide. The values of these variables were derived using an intensity-of-exposure algorithm, which incorporates information from an extensive literature review and pesticide field monitoring data (113-116).This is also the first epidemiological study of short-term high-level pesticide exposure events and ESRD. An additional strength of this study lies in its ability to evaluate pesticide use and ESRD among both males and females, whereas most occupational chemical exposure research has traditionally been conducted among predominantly male cohorts. Further, investigation of indirect exposures among wives who do and do not use pesticides permits assessment of the impact of non-occupational pesticide exposure on ESRD risk. The fact that almost all ESRD cases in the United States are captured in the USRDS reduces concerns about loss to follow-up or outcome misclassification. This research represents the first evaluation of the impact of long-term use of specific chemicals, short-term high level pesticide exposures, and indirect pesticide exposures on the incidence of ESRD among a large well-characterized cohort of male pesticide applicators and their wives.

As the first study to evaluate the effect of chronic exposure to a broad range of pesticides on incident ESRD, the proposed research has the potential to greatly improve our understanding of the health effects of direct use of and indirect exposure to specific pesticides. The population under study is a moderately exposed cohort of pesticide applicators and their spouses. Finding an association in this study could have a substantial public health impact not only for the 3.3 million farm operators and their families, but also for the 3 million migrant and seasonal farmworkers in this country (117) who often experience even higher levels of exposure. The outcome under study represents the most severe form of

renal impairment. Because ESRD is the final phase of a chronic disease that takes years to develop, identification of pesticides as risk factors could inform prevention strategies that would have implications for those at risk of developing CKD, as well as ESRD. The United States uses more than one billion pounds of conventional pesticides each year (40). Understanding the relationship between such heavily used chemicals and ESRD could help inform policy discussions about pesticides and safety regulations for agricultural workers in the US. Banning chemicals that are chronically and acutely nephrotoxic can improve population health without requiring behavior change. Findings from this study can also provide context for research on region-specific kidney disease epidemics concentrated in agricultural populations.

CHAPTER 2: RESEARCH DESIGN AND METHODS

Human Subjects

This research has been approved by the Institutional Review Board at the University of North Carolina (Reference ID: 13-2276)

Approach

We conducted a longitudinal study of the relationship between pesticide use and exposure and incident ESRD in a cohort of pesticide applicators and their spouses in NC and Iowa. This study made use of the extensive data on self-reported pesticide use and other pesticide exposures available through the Agricultural Health Study (AHS). AHS study data was linked with data from the United States Renal Data System (USRDS). These linked data were used to evaluate the relationships between long-term, high-level, and indirect pesticide exposure and ESRD.

Study Population

As the largest US cohort study of individuals working with pesticides, the Agricultural Health Study is an ideal resource for evaluating the impact of pesticides and other related agricultural hazards on disease incidence. Farmers and commercial pesticide applicators in Iowa and North Carolina who applied for a restricted-use pesticide license between 1993 and 1997 were asked to participate in the study. There are two pesticide application licensing categories: "private" (mainly farmers) and "commercial" (persons employed by pest control companies or by businesses that use pesticides) (118). Approximately 82% (N= 52,394) of eligible private applicators in these two states enrolled in the study. Additionally, 47% of eligible commercial applicators in Iowa enrolled (N=4,916). At enrollment, applicators provided information on lifetime pesticide use and pesticide use practices, demographic characteristics, lifestyle activities, farm information, and medical history in a self-administered questionnaire. Of enrolled

applicators, 44% also filled out and returned a take-home questionnaire with additional questions about medical history, pesticide use, and proximity of the participant's home and private well to pesticide mixing and application areas (1). Participants who did and did not return the take-home questionnaire were similar with regard to farming practices, medical history, and demographic characteristics, except age distribution; in both Iowa and North Carolina those who returned the take-home questionnaire were significantly older than those who did not (119). Additional information about enrollment of study participants is available in Alavanja et al's description of the Agricultural Health Study (26).

Seventy-five percent of married applicators had their spouse enroll (N=32,345) by filling out and returning a questionnaire during this same time frame (4). Less than one percent of spouses were male. The spouse questionnaire elicited similar information to the applicator questionnaire, with additional questions about farm work activities and household hygiene practices. Additional characteristics of the study population are provided in Table 2.1.

Table 2.1: Characteristics of Agricultural Health Study Participants

At enrollment	Private Applicators (N=52,394)		Commercial Applicators (N=4,916)		Spouses (N=32,345)	
	N	%*	N	%*	N	%*
Gender						
Male	51036	97	4712	96	219	1
Female	1358	3	204	4	32126	99
Race						
White	49762	97	4855	99	30921	98
Other	1514	3	26	1	552	2
State						
Iowa	31876	61	4916	100	21771	67
North Carolina	20518	39	0	.	10574	33
Education						
≤High School	29285	59	2197	46	12917	59
>High School	20708	41	2557	54	15177	41
Smoking Status						
Never Smoked	26937	53	2312	48	21997	72
Past Smoker	15514	31	1245	26	5324	17
Current Smoker	8047	16	1282	26	3179	10
Ever mix or apply Pesticides	50620	99	4475	92	17628	56
No. of years mix or apply						
Never	498	1	411	9	13759	51
1 year or less	1116	2	438	10	1259	5
2-5 years	5571	11	1235	27	3548	13
6-10 years	7469	15	912	20	2548	9
11-20 years	15987	33	1071	23	3079	11
21-30 years	11672	24	410	9	1618	6
30+ years	6494	13	124	3	1168	4
Deceased as of 12/31/12	6109	12	294	6	2706	6
As of October 2005 (end of Phase 2 data collection)						
Kidney Disorder						
Yes	354	1	28	1	214	1
No	52040	99	4888	99	32131	99
High Blood Pressure						
Yes	8637	16	870	18	5954	18
No	43757	84	4046	82	26391	82
Diabetes						
Yes	2429	5	188	4	1314	4
No	49965	95	4728	96	31031	96
Age	Mean	SD	Mean	SD	Mean	SD
At Enrollment	47.1	13.3	38	11.5	46.9	12.1
Age 12/12/11	60.8	12.0	53.3	10.8	60.9	11.5

SD= Standard Deviation

Inclusion/ Exclusion criteria:

Because we could not know exactly when pesticide exposure began, it would be impossible to know if pesticide exposure occurred before ESRD diagnosis for cases diagnosed prior to enrollment. In order to avoid interpretation issues related to reverse causality, we excluded cohort members who had an USRDS-confirmed ESRD diagnosis on or before the date of enrollment (i.e. prevalent cases). Additionally, we found that prevalent cases were younger, more likely to have had a transplant, more likely to be never-smokers, and had much longer survival times following ESRD diagnosis compared to those diagnosed after enrollment (i.e. incident cases). (Appendix 4). For these reasons, we excluded cohort members who had an USRDS-confirmed ESRD diagnosis on or before the date of enrollment. This resulted in exclusion of 11.5% of cases among applicators (N=42) and 20% of cases among spouses (N=25). Gender is an important confounder because pesticide exposure distribution and use practices vary widely by gender, and ESRD is more common among men. By restricting our analyses to exclude female applicators (3%) and male spouses (1%), we could minimize bias and improve internal validity with very little loss in precision.

Commercial applicators and private applicators in the AHS use similar pesticides, but commercial applicators report greater use (120) and higher prevalence of high pesticide exposure events compared to private applicators (121). With a higher distribution of pesticide use, commercial applicators are an important group to study, particularly because we were interested in whether ESRD risk increases with increasing cumulative exposure. Given the small number of commercial applicators in the applicator cohort, we did not have enough statistical power to analyze this group separately. Differences between commercial and private applicators (i.e. commercial applicators only enrolled in Iowa and on average younger than private applicators) could be accounted for in statistical analyses through adjustment by age and license type. Therefore, commercial applicators were included in analyses of chronic and short-term high-level pesticide exposures among applicators (Aim 1).

Outcome ascertainment

Outcome definition

Chronic renal failure is the final stage in the progression of chronic kidney disease and is characterized by severely limited kidney function that is irreversible. End-stage renal disease (ESRD) is defined as renal failure requiring dialysis or kidney transplant for survival (i.e. renal replacement therapy).

Case status

Data for the USRDS database are compiled from existing data sources including the Medicare Evidence form (CMS-2728), ESRD Death Notification form (CMS-2746), CMS Renal Management Information System, CMS claims data, CDC survey data (NHANES), and United Network for Organ Sharing (UNOS) transplant and wait-list data. Since 1988, the USRDS has tracked all ESRD cases with a few exceptions. First, reporting of dialysis initiation using the Medical Evidence form 2728 was not required for non-Medicare-eligible (i.e. those covered by private insurance or Veterans Affairs) patients prior to 1995; after 1995, form 2728 was required for all new ESRD patients regardless of Medicare status. Thus, it is possible that patients who were not Medicare-eligible between 1993 and 1995 are missing from our dataset. However, we expect few if any missing cases because diagnosis within 2 years after study enrollment was uncommon in this cohort; only 6% of cases in the study cohort were diagnosed within 2 years of enrollment. Second, patients under the age of 65 with pre-existing primary insurance payers are not eligible for Medicare entitlement until 90 days after initial ESRD diagnosis (27). Thus, it is possible that patients who die within that 90-day window may not be captured in the USRDS database. However, the Medicare Evidence form 2728 is required for all ESRD patients regardless of Medicare eligibility. There is no evidence that dialysis centers wait to submit the 2728 form for these patients until the 90 days have elapsed (Email from Paul Eggers, Director of Kidney and Urology Epidemiology at the National Institute for Diabetes and Digestive and Kidney Diseases, April 22, 2013). Therefore, potential

outcome misclassification due to missing data on ESRD patients not captured by the USRDS is likely to be trivial.

The USRDS database captures information on diagnosis date and dialysis modality, as well as demographic and comorbidity information, death date and cause of death. ESRD status of AHS study participants was determined using USRDS data.

Date of Diagnosis:

The first ESRD service date (FSD) was used to calculate age at ESRD diagnosis, which was used for modeling survival analyses. The FSD is derived by taking the earliest of:

- the date of the start of dialysis for chronic renal failure, as reported on the Medical Evidence report,
- the date of a kidney transplant, as reported on a CMS or UNOS transplant form, a Medical Evidence report, or a hospital inpatient claim, or
- the date of the first Medicare dialysis claim (27).

ESRD Case Numbers

The AHS-USRDS linkage identified 348 ESRD cases among private applicators, 17 cases among commercial applicators, and 132 cases among wives. After exclusion of prevalent cases, final numbers of cases for analyses were 308 among private applicators, 12 among commercial applicators, and 103 among wives. Though exclusion resulted in a considerable loss of cases for analysis, we could not include prevalent cases because we could not be sure that exposure occurred before diagnosis.

Non-ESRD Deaths

Death dates were obtained for all participants by annual linkage to the National Death Index.

Exposure ascertainment

Overview of Data Collection

Aim 1

On the enrollment questionnaire, applicators provided information on ever use of 50 pesticides, cumulative use of 22 of those pesticides, use of personal protective equipment, pesticide application methods, pesticide mixing status, equipment cleaning and repair methods, and frequency of medical visits due to pesticide use. On the take-home questionnaire (completed by 44% of applicators), applicators provided additional information on cumulative use of the remaining 28 pesticides. Pesticide use was identified through questions which asked participants whether they "personally mixed or applied" a specific pesticide. Chemical names and brand names were used in these questions. Cumulative use data includes number of years of use, average number of days per year of use, and decade of first use. Data were also collected on application methods and mixing practices for functional classes of pesticides, whether the applicator repairs his/her own pesticide application equipment, and use of personal protective equipment. These measures were used in conjunction with data from exposure monitoring studies to derive an exposure intensity score for each pesticide, which has been evaluated and refined since it was first developed for the AHS. Descriptions of the derivation and adjustment of the intensity score can be found in Coble et al (2011), Thompson et al (2010), and Dosemeci et al (2002) (114-116).

Aim 2

Spouses provided data on ever use of 50 specific pesticides, percent of time mixed any pesticide, percent of time applied any pesticide, and cumulative use of any pesticide. For spouses, cumulative use data on specific pesticides was not collected during the first phase of the AHS. Indirect pesticide exposures of interest included proximity of the home to pesticide mixing/application area, whether family members leave work boots on in the house after mixing/applying, whether contaminated clothes are mixed with the family wash, and applicator use of specific pesticides. Data were also collected on number

of days worked in the field during the last growing season, ever having an off-farm job, and number of hours per days spent in the sun during the growing season.

A list of the exposures evaluated and questions and response options associated with those exposures is provided in Appendix 5.

Rationale for use of pesticide exposure variables

In this study, we looked at pesticide use in several ways. Among applicators, cumulative use (lifetime days and intensity-weighted lifetime days) of specific pesticides allows us to look at a whether ESRD risk is associated with increasing duration and extent of exposure to specific pesticides. Analysis of specific pesticides as low level chronic nephrotoxins facilitates understanding of the effect of long term pesticide exposure on human kidneys. Pesticide poisoning is known to cause acute renal failure for certain pesticides. Little is known about the effect of pesticide poisoning on chronic disease. Analyses of high level exposure incidents among applicators provided information on the impact of acute pesticide exposure on the development of severe chronic renal disease.

Among wives, evaluation of cumulative use of any pesticides allowed us to look at the relationship between a gradient of exposure and incident ESRD. While evaluating pesticide use provides information about the role of direct contact with pesticides and ESRD, assessment of indirect pesticide exposures can elucidate other important pathways for incident disease. Farm wives may be exposed to pesticides in a variety of ways other than mixing and applying pesticides themselves. Pesticide residues tracked into the home on applicators' shoes and clothing represent an important route of exposure for family members who do not engage in agricultural work (122-124). Analyses of take-home exposures, including applicator work boot removal and washing contaminated clothing with the family laundry, can facilitate understanding of the role of take-home exposures in ESRD risk. Additionally, analyzing applicator use as a potential indirect exposure mechanism for wives allowed us to assess whether applicator use has an impact on the health of their wives. Studies have also shown that proximity of the home to pesticide application areas is positively correlated with house dust pesticide levels and urinary

pesticide metabolite levels among family members who do not work in agriculture (23, 24). Elevated ESRD risk among wives whose homes are closest to the location of mixing and applying may suggest an adverse effect of pesticide drift or drinking water contamination. Evaluation of indirect exposures among non-pesticide-applying AHS wives provided a more complete picture of the patterns of ESRD risk among wives of pesticide applicators. We also considered the number of days wives reported working in the fields during the last growing season, the number of hours spent in the sun per day during the growing season, and ever having worked a job off of the farm. We assumed that spending time in the fields or outside during the growing season could potentially increase exposure through drift or contact with sprayed plants. We looked at whether participants had a non-farm job because those who have always worked on the farm likely have greater lifetime pesticide exposure opportunity than those who are away from the farm for periods of time, though we acknowledge that this is a crude measure of exposure. Lastly, we evaluated spouse use of pesticides in the home, lawn or garden in relation to ESRD risk.

Evaluation of use of specific pesticides allows for comparison of the relative risk of ESRD for users of a given pesticide compared to those who do not report using that pesticide. For analysis of a given pesticide, records with missing use information for that pesticide were excluded.

Covariate Assessment

Data collection

Covariates of interest were identified through a review of the literature on predictors of pesticide use and risk factors for ESRD. For applicators, the enrollment questionnaire captured data on age, gender, education level, race, ever diagnosis of diabetes and smoking status. Body mass index (BMI), hypertension, and NSAID use were only captured on the take-home questionnaire. BMI was imputed by AHS study staff for those who did not return the take-home questionnaire. Data on all spouse covariates of interest were collected using the spouse questionnaire.

Missing covariate information

Data were complete for age, gender, and state. Diabetes data were missing for 8% of the cohort (14% of cases and 8% of non-cases). Some enrolled applicators and spouses provided information on having received a diagnosis and age at diagnosis for diabetes on follow-up questionnaires administered during Phase 2 (1999-2005) and Phase 3 (2005-2010) of the Agricultural Health Study. These data were used to fill in missing values for diabetes if the reported age at diagnosis was the same as or lower than the age at enrollment into the study. This process resulted a reduction in missing diabetes data to only 5%, and the total prevalence of diabetes in the cohort changed very little (increased from 2.8% to 3.1%). After filling in missing values, data were missing for 8.4% of cases and 5.1% of non-cases.

Analytical Plan

Use of Cox proportional hazards models (Aims 1 and 2)

Cox proportional hazards models were used to estimate hazard ratios for all exposures of interest. Other modeling strategies were considered, including multilevel modeling, which has been shown to correctly model correlated error for multiple correlated exposures, such as pesticide use. However, the use of this method would result in a substantial loss of data for each model, due to the fact that individual records are likely to have missing data for at least one pesticide in a given pesticide class. In this study of

a rare outcome, Cox models are preferable to other modeling options because all observations with exposure and outcome data are included in the estimation of the hazard ratio, lending increased power to statistical analyses. Cox models assume the hazard function varies over time, proportionally for exposed and unexposed groups. Our study data are more likely to fit this assumption than the stricter constant hazard assumption of the Poisson regression model. Chronological age was chosen over time-on-study for the time scale because age is a strong confounder of the association between pesticide exposure and ESRD. Use of age as the time scale allowed for adjustment of this confounder.

Model Building Strategy

For Aim 1, the association between pesticide use and ESRD was first evaluated in adjusted Cox hazards models with an ever/never exposure variable for each of the 50 pesticides. Participants provided information on ever use of 50 specific pesticides along with information on years of use (duration) and average days per year of use (frequency) for 22 of the pesticides on the enrollment questionnaire. The question on ever use was repeated and duration and frequency of use data were obtained on the take-home questionnaire for the remaining 28 pesticides. To facilitate comparison of the same populations for ever/never and cumulative use analyses, we elected to use enrollment questionnaire data for ever use of 22 pesticides and take-home questionnaire data for ever use of the other 28 pesticides. Pesticides ever used by less than 5 cases were not analyzed in ever use or cumulative use analyses. As such, the fumigant aluminum phosphide, the fungicide ziram, and the insecticide trichlorfon were not analyzed due to an insufficient number of cases reporting use of those pesticides.

Lifetime-days of use of specific pesticides was calculated by multiplying the midpoints of the questionnaire categories of number of years an applicator personally applied or mixed a specific pesticide by the midpoints of the categories of number of days in an average year an applicator personally applied or mixed that pesticide. Lifetime-days of use was then multiplied by an exposure intensity score for each pesticide (114). The intensity-weighted exposure metric was chosen over cumulative lifetime-days of use as the main exposure metric for each pesticide because it is thought to more closely approximate true

lifetime exposure. Using intensity-weighted lifetime-days of exposure vs. cumulative lifetime-days of exposure resulted in <1% reduction in sample size for all pesticides except for chlorpyrifos, for which there was a 14% reduction in sample size.

At enrollment, applicators also provided information on the number of hospitalizations and visits to a medical professional as a result of pesticide use. The take-home questionnaire elicited additional information about diagnosis of pesticide poisoning and a history of unusually high personal exposure to pesticides in general. Cox models were also used to evaluate ESRD risk in relation to these pesticide exposure measures.

For Aim 2, ever/never exposure to 50 pesticides and cumulative exposure to any pesticides was evaluated for association with ESRD in Cox models among wives who reported prior agricultural pesticide use. Lifetime-days of use of pesticides in general was calculated in the same way as was done for the applicators. Among wives who do not apply pesticides, indirect cumulative exposure to specific pesticides was evaluated using the cumulative pesticide use information of their private applicator husbands. Duration of potential pesticide exposure was based on the number of years couples lived together prior to enrollment and the frequency and duration of use by the applicator. In Phase 3 of the AHS (2010-2012), applicators and their spouses provided information about the number of years that they had lived together up to that point. Of 24,172 applicators and 19,959 spouses, 98% of spouses and 70% of husbands provided this information. Spouse report was used as the main value, except where spouse data were missing, in which case the applicator husband's report was used. Using the date of enrollment, we calculated the number of years that husband and wife lived together prior to enrollment. We then calculated the median number of years that spouses were living with their husbands for each age stratum based on their age at enrollment. These median values were then imputed for spouses who were not interviewed at Phase 3. The date that spouses began living with their husbands was defined as the enrollment date minus the number of years that spouses lived with their husbands prior to enrollment.

Several questions from the enrollment and take-home questionnaire were used to calculate potential exposure that the wives experienced based on their husbands' use of specific chemicals. Husbands reported the first decade that they used each chemical. In order to define a 'date of first use,' we assumed that the husbands began using each chemical at the midpoint of the reported decade of first use. On average, 6% of pesticide users did not report decade of first use. Where decade of first use was missing, date of first use was defined as enrollment date minus the number of years that the husbands reported using each chemical. Imputation of these dates shifted the range, but not the median, of the date of first use for each chemical. In situations where data were missing for both decade of first use and years of use, the date of first use was set to missing. The end date of the husbands' use was defined as the date of first use (as defined above) plus the number of years that husbands reported using each chemical, truncated at the applicator's date of enrollment.

For pesticides that were banned prior to enrollment (i.e. chlordane, heptachlor, aldrin, dieldrin, DDT, and toxaphene), the process of determining date of first use was slightly different. We assumed that applicators used these pesticides for up to 5 years after the date that use was banned. For these pesticides, if decade of first use was missing, the date of first use was set as July 1st of the year the pesticide was banned + 5 years, minus the number of years of use. For example, DDT was banned in 1972, so if decade of first use was missing, the date of first use was set as July 1, 1977 minus the number of years of use. The end date was the earliest of: 1) the date of first use plus the number of years of use, or 2) July 1st of the year the pesticide was banned + 5 years.

Finally, the number of years that wives could be exposed to each chemical was defined as the difference between the later of the date that spouses began living with their husbands (as defined above) or the date of his first use, and the end date. Lifetime-days of exposure to their husband's use of each chemical was defined as the number of years that wives could be exposed to each chemical multiplied by the average number of days per year that husbands reported using that specific chemical. Of the 50 pesticides evaluated, only 22 had at least three cases in each exposure stratum.

Table 2.2. shows several illustrative examples of the calculation of wives' lifetime-days of exposure to use of specific pesticides by the husbands.

Table 2.2: Examples of calculations of wives' cumulative exposure to husband's use of specific pesticides

	Applicator husbands			Wives					
Partic-ipant	Decade of first use	Number of years of use	Enrollment date	Estimated date that wife began living with husband	Date of first potential exposure	End date of potential exposure	Number of years exposed	Avg number of days per year husband uses chemical	Lifetime-days of wives' exposure
A	1970s	10	1/1/1994	7/1/1980	7/1/1980	7/1/1985	5	50	250
B	1980s	25	1/1/1994	7/1/1972	7/1/1985	1/1/1994 (enrollment date)	8.5	20	170
C	Missing	15	1/1/1994	7/1/1970	(1/1/1994 – 15 years) = 1/1/1979	1/1/1994	15	30	450
D (for **DDT,** banned in 1972)	Missing	15	1/1/1994	7/1/1965	(7/1/1977 – 15 years) = 7/7/1962	7/1/1977	12	10	120

Associations between indirect cumulative pesticide exposure and ESRD were assessed in Cox hazard models adjusted for potential confounders. Cox models were also constructed with each of the following other pesticide exposure measures as the main exposure variable: number of days worked in the fields during the last growing season; number of hours per day spent in the sun during the last growing season; ever had an off-farm job; proximity of home to location where pesticides are applied; distance between private well and pesticide mixing area; work boots left on in house; contaminated clothing mixed with family wash; number of days per year wife washed contaminated clothing. ESRD risk associated with these measures was first evaluated among all wives, adjusting for pesticide use. We also evaluated these associations among the sub-cohort of wives who reported no prior pesticide use.

Models were only constructed for pesticides with 3 or more exposed cases in each exposure category. While Firth's estimation method has been found to produce more accurate estimates in Cox models than the classical Cox partial likelihood when case numbers are small, the partial likelihood estimator appears to produce similar results to the Firth-estimated results when there are more than 8 cases in each stratum (125). Therefore, we used Firth's penalized likelihood to estimate hazard ratios when there were less than 8 cases per strata.

The following general model -building approach was applied for Aim 1 and Aim 2.

Bivariate relationships

Bivariate distributions were explored to assess relationships between exposures, covariates, and outcomes (i.e. contingency tables). These analyses informed the choice of an appropriate categorization for each variable that allows for flexibility in exposure-response relationships while maximizing parsimony, model fit, and ease of interpretation. For aim 1, intensity-weighted cumulative use of specific pesticides was categorized into three or four levels depending on the number of cases who reported using each chemical. For pesticides used by ≥15% of cases, we categorized intensity-weighted lifetime-days of use into tertiles with non-users as the referent group. For less frequently used pesticides, intensity-

weighted lifetime-days of use were split at the median (<median vs. ≥ median), with non-users as the referent group. For wives' cumulative exposure to husbands' use of specific chemicals, we used the latter 3-level categorization for all pesticides.

Confounding assessment

Directed Acyclic Graph (DAG) analyses were informed by the literature review described in Appendix 8. Minimally sufficient adjustment sets (MSAS) were identified through DAG analysis for Aims 1 and 2. For Aim 1, the MSASs were equivalent for each DAG, with state, age, gender, and education identified as potential confounders. A variation of the DAG identified exposure to solvents and silica as potential confounders, but these were not adjusted for in our analyses because they were not associated with ESRD. For Aim 2, the MSAS for non-application exposures also included spouse ever use of pesticides in general. Adjustment for gender is addressed through restriction: female applicators were excluded from analyses of AHS applicators, and male spouses were excluded from analyses of AHS spouses. All analyses were adjusted for age: models in Aim 1 and Aim 2 were adjusted for age via use of age as the time scale and age was used as a standardization factor in SIR analyses. Rationale for non-adjustment of other risk factors for ESRD are described in the methods sections of Aims 1 and 2, and in the literature review provided in Appendix 6.

Stratified Analyses

In order to evaluate whether patterns in associations with specific pesticides were consistent across state, results for cumulative use of specific pesticides were stratified by state for pesticides with at least 5 cases in each exposure stratum. The presence of a statistically significant interaction at $\alpha = 0.1$ indicates that the association between use of specific pesticides and ESRD risk differs by state. Additionally, patterns of exposure were reviewed for consistency across state.

Sensitivity Analyses

To assess potential variability in results when commercial applicators were included in the analyses vs. analyses of private applicators only, Aim 1 analyses were run using a dataset which includes commercial applicator data and then in a dataset that excludes commercial applicator data.

Individuals with earlier stages of renal disease may have limited their pesticide use prior to enrollment in the AHS. If this is the case, estimates of the association with pesticide use will be biased towards the null (i.e. healthy worker survivor effect) (126). To evaluate the potential impact of this bias, we ran analyses removing cases that were diagnosed within five years after enrollment and moving the enrollment date for non-cases forward by five years. Depending on the severity and rate of progression of disease, it can take anywhere from less than a year to 45 years for an individual to progress from earlier stages of disease to progress to end-stage renal disease (127). In this sensitivity analysis, we assumed that individuals diagnosed within five years after enrollment were likely experiencing the health effects of later stages of chronic kidney disease (49) before or around the time of enrollment, which may have limited their pesticide applying activity.

Correlated exposures

We expected to see correlations within the pesticide use data because chemicals are often used in combination in pest management and chemicals that are taken off the market may be substituted with other chemicals over time. However, large correlations between pesticide variables were not expected to be very common. The highest observed correlation among 50 pesticides was 0.37 for 2,4,5-T and 2,4,5-TP in an AHS study of asthma among 19,704 male farmers (31). And, in an analysis of organophosphate use among private pesticide applicators, only 17 of 190 possible organophosphate pairs had correlation coefficients greater than 0.2 (128). To assess potential confounding by other pesticides, we examined pairwise correlations between pesticides that were strongly (HR in any strata $\geq$1.5 or $\leq$0.65) or significantly associated with ESRD in single pesticide adjusted ever/never or exposure-response models. For pesticides with a Spearman correlation coefficient $\geq$0.3, we constructed models with both pesticides,

using the ever/never or intensity-weighted variable categorizations used in the main analyses. For pesticides that were correlated with more than one pesticide, we first evaluated each pesticide pair and then added correlated pesticides one at a time into subsequent models. We assessed model fit using Akaike information criteria (AIC) and selected that with the lowest AIC as the final model.

Model diagnostics

Adherence to Cox hazard model assumptions. Proportional hazards assumptions were met for exposures and covariates of interest.

Test for trend

Exposure-response trends were evaluated by including the midpoint of each exposure category as a continuous variable in regression models and testing for statistical significance of the slope, as has been done in prior AHS studies (129, 130).

Adjustment for multiple comparisons

The chance of making a Type 1 error (incorrect rejection of the null hypothesis) increases with the number of comparisons. However, adjustment for multiple comparisons increases the chance of making a Type 2 error (accepting the null when there is a true effect) (131). In this first investigation of the effects of long term and short term high level pesticide exposure on ESRD, it was preferable not to adjust for multiple comparisons.

Standardized Incidence Ratios (Aim 3)

The standardized incidence ratio analysis aims to assess whether ESRD risk is elevated among a group of people highly exposed to pesticides compared to the general population. Using linked data from the USRDS, we were able to identify virtually all incident ESRD cases in the AHS cohort. Population counts of ESRD by age, state, sex, race, and year are readily available through the USRDS Render system. ESRD incidence proportions were calculated for the general population, stratified by age category, sex, race, and state, using census data for rate denominators. Standardized incidence ratios were

calculated using indirect standardization, with the ESRD incidence in the general populations of Iowa and NC as the standard or reference population. Byar's approximation to the exact Poisson test was used to calculate 95% 2-sided confidence intervals (132).

If pesticide use truly increases the risk of ESRD, we would expect to see an elevated incidence of ESRD among an agricultural cohort highly exposed to pesticides compared to the general population. However, the AHS cohort has a more favorable risk factor profile with regard to ESRD incidence (e.g. lower prevalence of smoking, obesity, diabetes, and hypertension) compared to the general populations of Iowa and NC. Therefore, ESRD incidence was likely to appear lower in the AHS cohort compared to the general population. A typical indirect standardization process using general population data would not be able to take these risk factors into account. Consequently, ESRD risk would be overestimated among those without a given risk factor and underestimated among those with it. Standard techniques for indirect standardization would thus mask the impact of the varying incidence of key risk factors in the general population vs. the study population. In the context of this study, this masking effect would bias the results down towards the null, and potentially through the null. We addressed this bias by incorporating an adjustment factor for diabetes per methods described by Suta and Thompson (133). Estimates of annual population prevalence of diabetes by age, state, and sex were obtained from the Behavioral Risk Factor Surveillance Survey (BRFSS) (134). Risk ratio estimates for diabetes (RR) were obtained from Brancati et al (1997) (135).

Results Interpretation

Though statistical significance testing may be useful for obtaining a summary measure of the exposure-response relationship between cumulative pesticide use/exposure and ESRD, the relative importance of particular findings in this study were based on the magnitude of the hazard ratios, patterns observed in exposure-response analyses, and consistency across states and/or across aims.

Power

Pre-analysis power calculations were done to evaluate power for each aim, given a fixed sample size, but allowing for flexibility in the percent exposed to specific pesticides. We assumed an average of 15 years of follow-up and ascertained exposure prevalences from published AHS studies. Power calculations are presented at $\alpha = 0.05$. Analyses were conducted in SAS 9.2 using the PROC POWER procedure.

Table 2.5: Power calculations for Aim 1 and Aim 2

Analysis	**N**	**Percent exposed**	**Survival**	**At least 80% power to detect an HR of:**
Aim 1: Applicators				
Ever use	55000	5/10/20/50	0.993	1.7/1.5/1.4/1.35
Use of 22 pesticides on enrollment questionnaire	Same as above			
Use of 28 pesticides on take-home questionnaire	22000	5/10/20/50	0.992	2.0/1.75/1.6/1.5
Medical visit	55000	7	0.993	1.95
HPEE	22000	14	0.992	1.7
Poisoning	22000	2	0.992	2.56
Aim 2: Wives				
Pesticide use (among wives who applied pesticides)	~16,000	5/10/20/50	0.996	2.68/2.24/1.96/1.86
Applicator ever use (among wives who did not apply pesticides)	~13,000	5/10/20/50	0.996	2.87/2.39/2.08/1.95
Work boots left on in home	~31,000	35	0.996	1.63
Contaminated clothes washed with family wash	~31,000	11/20	0.996	1.87/1.71
Home is less than 100 yds from pesticide application	~31,000	52	0.996	1.59

Power was found to be limited for some of the proposed analyses, particularly for evaluation of pesticide poisoning and washing of contaminated clothing. However, because these analyses are important for elucidating the wide range of opportunities for pesticide exposure, and the role of different sources of pesticide exposure in ESRD risk, we decided to pursue evaluation of these factors.

Strengths and Limitations

This study is the first and only prospective study of specific types, levels and routes of pesticide exposure and any type of kidney disease. Pesticide use and exposure information were obtained in a cohort study specifically designed to evaluate health effects of pesticide exposure, with data on use of 50 pesticides. Variability in cumulative use and types of pesticides used facilitated evaluation of the role of specific pesticides and exposure-response trends in ESRD risk. The large size of the cohort provided sufficient power to analyze categories of pesticide use and exposure, where other studies have only evaluated dichotomous measures of these factors. Enrollment from two states permitted state-stratified analyses, which can be used to compare direction of effect of specific pesticides. We had complete case ascertainment for the outcome of interest and reliable data on the first ESRD service date. Recall bias with regard to the exposure is minimized because of the prospective cohort study design, and reliability of reported pesticide use is satisfactory (136).

The most important limitation was the small number of cases that we had for several analyses, most notably in analyses of pesticide use by state (Aim 1), analyses of wives' ever use of specific chemicals among wives who used pesticides and analyses of indirect exposures among wives who did not apply pesticides (Aim 2), and SIR analyses by race (Aim 3). We used Firth's penalized likelihood to address any instability in Cox models due to low numbers in exposure strata for Aims 1 and 2. In Aim 3, though we could review distributions of cases by race, we could not standardize by race, an important risk factor for ESRD. Despite these limitations, analyses provided novel information on the relationship between pesticide use/exposure and ESRD and informed directions for future research. Additional limitations are described in the discussion sections of each study aim, and in the summary chapter.

CHAPTER 3: Pesticide use and risk of end-stage renal disease among licensed pesticide applicators in the Agricultural Health Study.

Introduction

In 2011, over 600,000 Americans were receiving treatment for end-stage renal disease (ESRD). Little is known about the impact of environmental and occupational factors on risk of ESRD. Some organophosphate (8), organochlorine (9), carbamate (10), and pyrethroid (11) insecticides and triazine herbicides (137) have been shown to cause renal damage and dysfunction in experimental animal models, and case reports of both fatal and non-fatal pesticide poisoning have described nephrotoxic effects of a variety of pesticide classes (13-17). Yet, the impact of long-term pesticide exposure on human kidney function remains largely unknown. Studies conducted in El Salvador, Nicaragua, and Sri Lanka indicate an elevated prevalence of chronic kidney disease among agricultural workers (18-21) compared to those who have never worked in agriculture; pesticide exposure is postulated to be a contributor to kidney disease in these regions, but existing evidence has not confirmed this hypothesis (18-20). To our knowledge, the only study to assess agrochemical exposure and ESRD found self-reported work in a place with frequent or daily exposure to insect or plant spray to be associated with increased ESRD risk (22). These studies lack specificity with regard to pesticide type and have not been able to adequately assess the long-term effects of chronic or acute pesticide exposure on ESRD risk.

The Agricultural Health Study (AHS) is the largest prospective study of pesticide applicators in the United States. Linking the AHS to the United States Renal Data System (USRDS) provides a unique opportunity to evaluate the relationship between pesticide use and exposure and ESRD risk. Using this linkage, we evaluated associations between chronic and acute pesticide exposure and ESRD risk.

Methods

Population and case definition:

The AHS recruited private pesticide applicators (mainly farmers) (N= 52,394) in North Carolina and Iowa and commercial pesticide applicators (N=4,916) in Iowa who applied for or renewed a restricted-use pesticide license between 1993 and 1997. Approximately 82% of eligible private applicators and 47% of eligible commercial applicators enrolled in the study. At enrollment, applicators provided information on lifetime pesticide use and pesticide use practices, demographic characteristics, lifestyle activities, farm information, and medical history in a self-administered questionnaire (26). Of enrolled applicators, 44% also completed a take-home questionnaire with additional questions about medical history and pesticide use (119). Questionnaires are available on the AHS web site: http://aghealth.nih.gov/collaboration/questionnaires.html.

We identified ESRD cases diagnosed between study enrollment and end of follow-up (December 31, 2011) through linkage with the USRDS. The USRDS collects data on all ESRD cases in the United States through Medical Evidence Form CMS-2728, which is required for all new ESRD patients, regardless of Medicare eligibility. The USRDS derives the first ESRD service date (FSD) by taking the earliest of: a) the date of the start of dialysis for chronic renal failure, as reported on the Medical Evidence form, b) the date of a kidney transplant, or c) the date of the first Medicare dialysis claim (27). The FSD was used to estimate age at ESRD diagnosis. Date of death was obtained from state mortality files and the National Death Index. Because the distribution of ESRD risk factors differs by gender, and because few applicators were female, we excluded female applicators from this analysis (N= 1,562; 2.7%). We also excluded applicators under age 18 (N=127; <1.0%) and ESRD cases diagnosed prior to enrollment (N=42; 11.5% of cases). This left us with 55,580 participants for analyses of enrollment questionnaire variables, and 24,565 participants for analyses of take-home questionnaire variables.

Exposure assessment

Participants provided information on ever use of 50 specific pesticides along with information on years of use (duration) and average days per year of use (frequency) for 22 of the pesticides on the enrollment questionnaire. The question on ever use was repeated and duration and frequency of use data were obtained on the take-home questionnaire for the remaining 28 pesticides. We evaluated ESRD in relation to ever use of these 50 specific pesticides, using enrollment questionnaire data for 22 pesticides and take-home questionnaire data for the other 28 pesticides.

For specific pesticides, an intensity-weighted exposure metric was generated by multiplying lifetime-days of use (product of duration and frequency of use) by an intensity score. This intensity score accounts for differences in exposure resulting from variation in pesticide application methods, repair of pesticide application equipment, and use of personal protective equipment (114). We used the intensity-weighted lifetime-days as our primary exposure metric. Due to the small number of cases, we used the distribution of use among cases to create cut-points for intensity-weighted lifetime use of specific pesticides. For pesticides used by ≥15% of cases, we categorized intensity-weighted lifetime-days into tertiles with non-users as the referent group. For less frequently used pesticides, intensity-weighted lifetime-days of use were split at the median (<median vs. ≥ median), with non-users as the referent group. Analyses were restricted to pesticides for which there were at least 5 cases in each exposure stratum.

To assess overall pesticide use, we evaluated risk related to duration, frequency and lifetime-days of use of any pesticide. Cumulative lifetime-days of use was categorized into quartiles, and duration and frequency of use were categorized into three levels (≤ the lowest category of use (referent), > lowest category of use to the median value, and > median).

Participant report of medical visits due to pesticide use (enrollment questionnaire), unusually high personal exposure to any pesticide (take-home questionnaire), and doctor-diagnosed pesticide poisoning (take-home questionnaire) were evaluated in relation to ESRD risk.

Statistical Analysis

We used Cox proportional hazards models to calculate hazard ratios for risk of ESRD, using age as the timescale and adjusting for state as a covariate in all models. Person-time was accrued from the date of study enrollment until the earliest of ESRD diagnosis, death, or the end of study follow-up. Private and commercial applicators were analyzed together because there were too few ESRD cases among the latter to analyze them separately. Race and education level were identified as additional potential confounders through directed acyclic graph analyses (DAGs) and review of prior literature. Because adjustment for these factors did not substantially change hazard ratio estimates and power was reduced due to incomplete ascertainment of education and race data, we did not adjust for these factors in the final analyses. Diabetes and body mass index (BMI) were not adjusted for because we have no evidence that those conditions affected pesticide use or exposure prior to enrollment, and they may be on the causal pathway between pesticide use/exposure and ESRD (1, 2, 5). Hypertension was not adjusted for because it is largely asymptomatic and therefore is unlikely to have affected pesticide use practices.

To assess potential confounding by other pesticides, we examined pairwise correlations between pesticides that were strongly (HR in any strata $\geq$1.5 or $\leq$0.65) or significantly associated with ESRD in single pesticide adjusted exposure-response models. For pesticides with a Spearman correlation coefficient $\geq$0.3, we constructed models with both pesticides, using the intensity-weighted variables included in the main analyses. For pesticides that were correlated with more than one pesticide, we first evaluated each pesticide pair and then added correlated pesticides one at a time into subsequent models. We assessed model fit using Akaike information criteria (AIC) and selected that with the lowest AIC as the final model.

To assess linear exposure-response trends in intensity-weighted lifetime use, we used within-category medians as the score for each level of use for each chemical. Exposure–response trends were also evaluated for duration, frequency, and cumulative lifetime-days of use of any pesticide, and number of doctor visits related to pesticide use.

ESRD is the final stage of chronic kidney disease (CKD), which is often debilitating in later stages of the disease. Cases may have already experienced the effects of CKD prior to study enrollment, which could have influenced their pesticide use. If those with earlier stages of renal disease have reduced exposure due to modified application practices, effect estimates for specific pesticide use would be biased towards the null. This bias is commonly referred to as the healthy worker survivor effect (138). To evaluate the potential for this effect to influence our findings, we repeated analyses, excluding person-time for all participants for the first five years after enrollment under the assumption that ESRD cases diagnosed within 5 years following study enrollment likely had poor renal health at enrollment.

To evaluate whether patterns of association were consistent across states, we entered a product term for state into ever and lifetime pesticide use models for those pesticides for which there were at least 5 cases in each stratum of use in both states.

We used the AHS dataset releases P1REL201209, P3REL201209.00, and AHSREL201304.00. All statistical analyses were done using SAS v9.3 (Cary, NC).

Results

Of the 55,580 participants eligible for analysis, 320 (308 private and 12 commercial) were diagnosed with ESRD over an average 15.7-year follow-up period (incidence rate: 36.6 ESRD cases per 100,000 person-years). Among the subset of 24,565 participants who returned the take-home questionnaire, there were 136 cases (incidence rate: 35.1 ESRD cases per 100,000 person-years). ESRD incidence was significantly higher in North Carolina compared to Iowa, regardless of age, which follows the pattern of ESRD incidence in the general population (27). In age- and state-adjusted models, education level greater than high school and obesity at enrollment were associated with increased risk of ESRD (Table 3.1). Self-reported doctor diagnosis of diabetes, high blood pressure, myocardial infarction, heart disease, and kidney disease (not counting kidney stones) were significantly associated with increased risk of ESRD (data not shown). Applicator type, farm size, number of years living on a farm, smoking, and alcohol consumption at enrollment were not significantly associated with ESRD risk.

More than one doctor visit due to pesticide use and hospitalization due to pesticide use were both significantly associated with ESRD with a significant trend observed for increasing number of pesticide-related doctor visits (*p for trend*=0.038) (Table 3.2). ESRD risk was not associated with self-reported unusually high personal pesticide exposure or pesticide poisoning, though only 5 ESRD cases reported a pesticide poisoning diagnosis. No significant exposure–response relationships were observed for duration, frequency, or cumulative lifetime days of overall pesticide use (data not shown).

Use of several pesticides was associated with ESRD risk (Table 3.3). Of the 47 pesticides with sufficient cases for ever use analyses, the fungicide metalaxyl and three herbicides (paraquat, petroleum oil, and imazethapyr) were associated with significantly elevated risk of ESRD. Ever use of carbaryl was inversely associated with ESRD. The fumigant aluminum phosphide, the fungicide ziram, and the insecticide trichlorfon were not analyzed due to an insufficient number of cases reporting use of those pesticides.

In intensity-weighted cumulative use analyses, positive associations were observed primarily among herbicides. ESRD risk was associated with the highest tertile of intensity-weighted use of five herbicides: atrazine, metolachlor, alachlor, paraquat, and pendimethalin, compared to no use. We observed a significant (*p for trend* <0.05) monotonic exposure-response trend with increasing pesticide use levels for all of these herbicides.

Among non-herbicide pesticides, ESRD risk was associated with the highest tertile of metalaxyl (fungicide) use (HR = 1.92; 95% CI: 1.01, 3.66), with evidence of a positive exposure-response trend (Table 3.4). Associations for the insecticides coumaphos and parathion (organophosphates), aldicarb (carbamate), and chlordane (organochlorine) were elevated (i.e. >1.6), but did not reach statistical significance. A positive monotonic exposure-response trend was observed for chlordane, heptachlor (organochlorine), and coumaphos (organophosphate), and a possible threshold effect was observed for chlorothalonil and aldicarb.

In analyses of correlated pesticides, we found fourteen pesticide pairs had Spearman correlation coefficients ≥0.30. Adjustment for correlated pesticides resulted in reduced overall sample size due to

missing data for each chemical. Adjusted estimates were similar in magnitude and direction, but were less precise. Patterns of exposure-response also did not change. After adjustment for correlated pesticides, the association between ESRD risk and the top tertile of intensity-weighted use remained significant only for pendimethalin, and this association became significant for chlordane (HR=1.93; 95% CI: 1.01, 3.70). Estimates for atrazine, alachlor, metolachlor, and aldicarb remained elevated but were no longer significantly associated with ESRD risk after adjustment for correlated pesticides. Paraquat, petroleum oil, chlorimuron-ethyl, coumaphos, parathion, and aldicarb were not highly correlated with other pesticides (data not shown).

In the sensitivity analysis evaluating the potential for a 'healthy worker survivor effect', we excluded 53 cases that were diagnosed with ESRD within 5 years after enrollment and 277,900 person-years (Table 3.5). The greatest percent reductions of case numbers were observed in the 'None' use category for all pesticides. In general, associations for intensity-weighted lifetime-use were in the same direction and of similar magnitude compared to estimates in the main analyses. Of note, age- and state-adjusted estimates for the highest quantile of intensity-weighted chlordane (HR= 1.99; 95% CI: 1.07, 3.68) and coumaphos (HR=1.81, 95% CI: 1.03, 3.17) use became significant, and the inverse HR for carbaryl was no longer significant (Table 3.5).

Results did not change substantially when we restricted analyses to private applicators (data not shown). Thirteen pesticides for intensity-weighted lifetime use and 35 pesticides for ever use had 5 exposed cases in each exposure stratum in both states, and were therefore included in analyses of interaction with state. P-values for interaction with state were consistently > 0.10 for cumulative use and ever use (data not shown). Upon review of state-stratified estimates (Table 3.6), we observed general consistency in patterns of associations and overlap of confidence intervals. Positive associations were somewhat more pronounced in North Carolina vs. Iowa for the highest tertile of use of atrazine, alachlor, and metolachlor.

Discussion

To our knowledge, this is the first study to evaluate the association between ESRD risk and cumulative lifetime pesticide use. Among pesticide applicators in the AHS, we found significant positive associations between intensity-weighted use of several specific pesticides and ESRD; excluding cases that arose within 5 years after enrollment strengthened some of these associations, though estimates were less precise due to the reduction in sample size. This is also the first epidemiological study of ESRD risk associated with non-fatal pesticide poisoning, acute high-level exposure, and pesticide exposure requiring medical attention. Participants who reported doctor visits and hospitalization due to pesticide use had a significantly higher risk of ESRD diagnosis compared to those who did not, but we did not observe increased risk with applicator report of doctor-diagnosed pesticide poisoning or unusually high personal pesticide exposure.

Prior epidemiological research on pesticide exposure and kidney disease is limited. Results from several cross-sectional studies evaluating the relationship between agricultural work and CKD suggest a potential association between agricultural work, particularly field work, and CKD prevalence (19, 109), but studies of pesticide use and CKD have been inconclusive (19, 20, 139). A cross-sectional study evaluating relationship between non-specific cumulative pesticide use and serum creatinine levels observed a weak ($r = -0.24$), but significant, inverse correlation between cumulative pesticide exposure and serum creatinine (112). Though this inverse relationship would seem to suggest that pesticide exposure was associated with better kidney function, it is possible that those with higher serum creatinine values (i.e. less healthy individuals) work less and therefore use pesticides less heavily. The only study to assess the relationship between ESRD risk and agricultural exposures observed an increased risk of ESRD among a large health maintenance organization population in the San Francisco Bay area who reported that they worked in a place with "frequent or daily exposure to insect or plant spray" (unadjusted hazard ratio: 1.78; 95% CI: 1.36-2.34) (22). In contrast, results from our analyses of general pesticide use did not

show an association with ESRD; however the vast majority of participants in our study was exposed to at least one pesticide.

Epidemiologic studies of renal effects of specific pesticides are rare. Hernandez et al (2006) found no difference in serum creatinine levels among greenhouse workers with higher vs. lower levels of apparent cholinesterase inhibition (used as a marker for pesticide exposure) (112). Serum levels of several organochlorine insecticides among chronic kidney disease patients were inversely associated with kidney function, potentially indicating a renal filtration deficiency or a reduction in pesticide application activities (and thus exposure) due to illness (111). In our study, ESRD risk was elevated for three cholinesterase-inhibiting insecticides (the carbamate aldicarb and the organophosphates coumaphos and parathion), with a moderate positive trend observed for coumaphos. No associations were seen with organochlorine use, except for chlordane, which was significantly positively associated with ESRD risk after adjustment for correlated pesticides and in analyses excluding cases diagnosed within five years after enrollment. Glyphosate was recently banned in Sri Lanka due to its hypothesized association with kidney disease. However, studies leading to its ban have suggested that glyphosate exposure leads to renal failure only when combined with high-level exposure to heavy metals (140). We found no evidence of an association between ESRD risk and glyphosate exposure.

Experimental evidence supports our findings of positive associations with exposure to the herbicides atrazine, alachlor, paraquat, and pendimethalin and the fungicide metalaxyl. Glomerular lesions and renal tubular necrosis due to oxidative stress-induced cell damage have been observed in animal models with exposure to metalaxyl and paraquat (141, 142), and kidney damage and dysfunction have been observed in rats exposed to atrazine (137) and fish exposed to alachlor (143). There have been no reports of renal effects of pendimethalin among mammals; however, at least one formulation of pendimethalin contains monochlorobenzene as an inert ingredient, which has been shown to cause kidney damage in rats (144). Although we observed a positive exposure-response trend for metolachlor, we found no published studies implicating this chemical in renal dysfunction or oxidative stress pathways.

Sub-acute tubulointerstitial and glomerular damage, such as that observed in animal studies with prolonged low dosing of pesticides (79, 83), can initiate a feed-forward loop of kidney injury and progressive loss of function that leads to ESRD (55). Additionally, acute kidney injury, a frequent outcome of pesticide poisoning (46), is associated with increased risk of subsequent chronic kidney disease and ESRD (98, 99). Once kidney function has begun to decline, concentrating defects associated with kidney dysfunction can lead to increased concentrations of toxic chemicals in the compromised kidney, causing further damage. Thus, it is possible that short-term high level and/or chronic pesticide exposures alone or jointly could increase the risk of ESRD through one or more sub-acute (or sub-clinical) kidney insults.

A significant positive exposure-response trend was observed for pesticide-related doctor visits, and participants who reported being hospitalized due to pesticide use had three times the risk of ESRD compared to those who did not. These findings support the hypothesis that frequent and/or severe pesticide exposures may increase the risk of ESRD. We did not see an association between pesticide poisoning or self-reported unusually high pesticide exposure and ESRD; however, power to detect an association was limited because information for those exposures was available only for participants who returned the take-home questionnaire, and pesticide poisoning was a rare event in the cohort.

The study improves upon prior research in several ways. First, we were able to evaluate associations of ESRD risk with a wide range of specific chemicals that vary in toxicity and extent of use. Whereas prior studies were limited by small sample size and exposure to few chemicals, the large size of the AHS cohort allowed us to assess exposure-response trends for many individual pesticides. Also, use of a validated exposure-intensity metric (114) allowed for a better estimate of each participant's likely pesticide exposure as opposed to non-specific pesticide use. Until now, the relationship between acute pesticide exposures and kidney disease has been evaluated only with respect to the effects of pesticide poisonings. Here, we were able to evaluate measures of non-poisoning acute pesticide exposures, thereby providing an important contribution to the scientific literature regarding occupational risk factors for

kidney disease. Additionally, the fact that almost all ESRD cases in the United States are captured in the USRDS reduces concerns about loss to follow-up or outcome misclassification.

Because exposure data were collected prior to disease onset and ESRD diagnosis data were obtained from a third party linkage rather than participant report, any exposure misclassification due to self-report is likely to be non-differential with respect to the outcome, which would bias estimates towards the null. Additionally, evidence suggests that report of pesticide exposure by AHS participants is reasonably reliable (136) and plausible (145). The accuracy and reliability of reporting acute pesticide exposures has not been investigated.

Lifetime use estimates could also be biased if participants with prevalent pre-end stage kidney disease modified their pesticide application practices in the period prior to enrollment. This 'healthy worker survivor effect' is a common problem in occupational health studies, including among pesticide applicators (146), frequently biasing estimates toward the null (138). Results of our sensitivity analysis suggest a minimal potential impact of this effect on HR estimates. However, it is possible that unmeasured post-enrollment pesticide use may differ by case status; if recent exposures are stronger contributors to ESRD risk than pre-enrollment exposures, then our results would still be biased towards the null.

Conclusions

Our study provides evidence for an association between ESRD risk and chronic exposure to specific chemicals among pesticide applicators in Iowa and North Carolina. Results from this study also suggest that pesticide exposures resulting in medical visits increase the risk of incident ESRD, raising concerns that multiple high-level pesticide exposures may contribute to irreversible kidney damage and resultant disease. Efforts to better characterize the pathway between pesticide exposure and kidney disease should include assessments of earlier disease stages, rate of progression from CKD to ESRD, and other potential routes of pesticide exposure, such as spray drift and carry-home exposures. Caution should be taken in interpreting results of such studies when diagnosis dates or disease severity information is not available,

because the healthy worker survivor effect may bias estimates towards the null. Additional research is needed to confirm the findings of our study, given the limited research on the role of pesticide exposure in the development of renal disease.

Table 2.1: Association between ESRD and demographic and medical conditions among private and commercial applicators, adjusted for age and state, Agricultural Health Study (1993-1997*)*

Variable (at enrollment)		Non-cases (N= 55,260) **N (%)**	ESRD† Cases (N=320) **N (%)**	**HR (95% CI)**
State (where enrolled)*	Iowa	49764 (93.4)	272 (91.3)	
	North Carolina	2635 (4.9)	15 (5)	2.02 (1.61, 2.53)
Applicator type	Private	884 (1.7)	11 (3.7)	
	Commercial			1.06 (0.58 1.93)
Race/ethnicity	White, non-Hispanic	51619 (98.9)	275 (96.2)	
	Other	572 (1.1)	11 (3.8)	17.65 (5.58 55.88)
Education level	High school or less	20112 (84.8)	115 (87.1)	
	More than high school	3596 (15.2)	17 (12.9)	1.49 (1.14 1.95)
Number years lived or worked on a farm over the lifetime	0-20	23506 (98)	126 (96.2)	
	21-30	484 (2)	5 (3.8)	1.43 (0.62 3.28)
	>30			0.90 (0.46 1.75)
Number of days per month drink alcohol in the last year	0	35943 (65)	134 (41.9)	
	1-23	19317 (35)	186 (58.1)	0.86 (0.66 1.11)
	≥24	50575 (91.5)	308 (96.3)	0.78 (0.45 1.36)
Number of pack years smoked over lifetime	None	4685 (8.5)	12 (3.8)	
	1-11	48860 (99.9)	231 (98.7)	0.80 (0.57 1.13)
	12-30	55 (0.1)	3 (1.3)	1.23 (0.91 1.66)
	>30	22452 (41.7)	74 (24.3)	1.36 (0.97 1.91)
Body mass index (kg/m^2)	<25	31369 (58.3)	231 (75.7)	
	25-29.99	2181 (10)	10 (7.9)	1.26 (0.89 1.79)
	≥30	2928 (13.4)	13 (10.2)	2.00 (1.37 2.93)

ESRD = end-stage renal disease.
*Adjusted for age only.

Table 3.2 Association between ESRD risk and pesticide poisoning, unusually high pesticide exposure events, and pesticide exposure requiring medical attention, adjusted for age and state, among male pesticide applicators, Agricultural Health Study (1993-1997)

Variable		Non-cases (N= 55,260) N (%)	ESRD Cases (N=320) N (%)	HR (95% CI)	P for trend
Number of times ever visited a medical doctor due to pesticide use	None	49764 (93.4)	272 (91.3)		
	Once	2635 (4.9)	15 (5)	1.07 (0.64, 1.8)	
	More than once	884 (1.7)	11 (3.7)	2.13 (1.17, 3.89)	
					0.0384
Ever hospitalized due to pesticide use	No	51619 (98.9)	275 (96.2)		
	Yes	572 (1.1)	11 (3.8)	3.05 (1.67, 5.58)	
Ever experienced unusually high personal pesticide exposure*	No	20112 (84.8)	115 (87.1)		
	Yes	3596 (15.2)	17 (12.9)	1.08 (0.65, 1.81)	
Ever diagnosed with Pesticide poisoning*	No	23506 (98)	126 (96.2)		
	Yes	484 (2)	5 (3.8)	1.59 (0.65, 3.89)	

ESRD = end-stage renal disease
* Question asked only on the take-home questionnaire: N (non-cases) = 24,429 and N (cases) =136.

Table 3.3: Association between ESRD risk and ever use of specific pesticides, adjusted for age and state, among male pesticide applicators, Agricultural Health Study (1993-1997)

Pesticide	Exposed Non-cases N (%)	Exposed ESRD Cases N (%)	HR (95% CI)
FUMIGANTS			
Methyl Bromide	8108 (15.2)	61 (20.3)	0.8 (0.59, 1.09)
Carbon tetrachloride/carbon disulfide (80/20 mix)*	1041 (4.4)	5 (3.9)	0.55 (0.23, 1.36)
Ethylene Dibromide*	1000 (4.2)	9 (7.1)	1.26 (0.62, 2.54)
FUNGICIDES			
Benomyl*	1981 (8.4)	14 (11)	0.97 (0.54, 1.73)
Chlorothalonil	4530 (8.5)	43 (14.5)	1.38 (0.98, 1.94)
Captan	5122 (10.4)	17 (6.9)	0.67 (0.41, 1.1)
Maneb*	1918 (8.1)	16 (12.7)	1.07 (0.61, 1.87)
Metalaxyl*	4570 (19.3)	38 (29.7)	1.56 (1.02, 2.39)
HERBICIDES			
Phenoxy herbicides			
2,4-D	40289 (75.4)	212 (70.4)	0.86 (0.67, 1.11)
2,4,5-T*	4361 (18.5)	27 (20.9)	0.72 (0.47, 1.11)
2,4,5-TP*	1220 (5.2)	11 (8.5)	1.26 (0.68, 2.34)
Triazine herbicides			
Atrazine	37284 (69.5)	214 (69.5)	1.19 (0.93, 1.53)
Cyanazine	20997 (42)	90 (34.5)	1.03 (0.78, 1.37)
Metribuzin*	8867 (37.5)	47 (35.9)	1.22 (0.83, 1.8)
Dinitroaniline herbicides			
Pendimethalin*	9135 (38.5)	48 (36.4)	1.19 (0.83, 1.69)
Trifluralin	26521 (53)	123 (47.3)	1.01 (0.78, 1.3)
Chlorocetanilide herbicides			
Metolachlor	23454 (46.9)	122 (45.5)	1.28 (1, 1.63)
Alachlor	26599 (53.1)	151 (55.9)	1.17 (0.92, 1.49)
Other herbicides			
Dicamba	25385 (51)	99 (37.6)	0.87 (0.65, 1.16)
Chlorimuron-ethyl*	7774 (32.7)	40 (30.8)	1.22 (0.84, 1.77)
EPTC	10312 (21)	38 (14.9)	0.98 (0.69, 1.41)
Paraquat*	4067 (17.2)	34 (26)	1.55 (1.02, 2.34)
Petroleum Oil*	5086 (21.6)	36 (27.7)	1.63 (1.11, 2.41)
Imazethapyr	21469 (43.4)	92 (35.1)	1.46 (1.08, 1.99)
Glyphosate	40684 (75.8)	222 (72.8)	0.83 (0.64, 1.07)
Butylate*	6278 (26.6)	27 (20.9)	0.85 (0.55, 1.32)

Table 3.3 continued

Pesticide	Exposed Non-cases N (%)	Exposed ESRD Cases N (%)	HR (95% CI)
INSECTICIDES			
Organochlorine insecticides			
Aldrin*	3865 (16.4)	36 (29.3)	1.21 (0.8, 1.84)
Chlordane*	4425 (18.8)	34 (26.8)	0.95 (0.64, 1.41)
DDT*	5217 (22.1)	48 (37.8)	0.82 (0.57, 1.20)
Heptachlor*	2763 (11.7)	27 (21.3)	1.39 (0.88, 2.19)
Toxaphene*	2605 (11.0)	19 (15.2)	0.88 (0.54, 1.44)
Lindane*	3023 (12.9)	16 (12.8)	0.87 (0.51, 1.47)
Dieldrin*	860 (3.7)	8 (6.5)	0.93 (0.45, 1.92)
Organophosphate Insecticides			
Terbufos	18896 (37.8)	88 (34.1)	1.09 (0.83, 1.42)
Fonofos	10560 (21.2)	34 (13.3)	0.72 (0.49, 1.06)
Chlorpyrifos	22092 (41.3)	100 (32.9)	0.82 (0.64, 1.04)
Malathion*	14953 (63.1)	85 (66.4)	1.04 (0.72, 1.51)
Parathion*	1819 (7.8)	16 (12.9)	1.22 (0.71, 2.09)
Diazinon*	5044 (21.4)	25 (19.8)	0.75 (0.48, 1.17)
Phorate*	6801 (28.8)	37 (29.8)	1.04 (0.69, 1.57)
Coumaphos	3933 (8.1)	30 (11.8)	1.45 (0.99, 2.13)
Dichlorvos	4831 (9.8)	23 (9.2)	1.11 (0.72, 1.72)
Pyrethroid insecticides			
Permethrin (for crops)	7217 (14.7)	36 (14.1)	1.15 (0.81, 1.64)
Permethrin (animals)	6096 (12.2)	26 (10.2)	1.38 (0.91, 2.08)
Carbamate insecticides			
Carbofuran	13198 (26.6)	67 (26.4)	0.83 (0.63, 1.1)
Carbaryl*	10180 (43)	57 (44.5)	0.66 (0.45, 0.97)
Aldicarb*	1787 (7.6)	15 (11.7)	1.61 (0.9, 2.87)

* Indicates pesticide with duration and frequency information only available on the take-home questionnaire: N (non-cases) = 24,429 and N (cases) =136.

Table 3.4: Intensity-weighted lifetime-days of use of specific pesticides and ESRD risk, adjusted for age and state, among male pesticide applicators in the Agricultural Health Study (1993-1997)

	Intensity-weighted lifetime-days	Exposed Non-cases N=55,260 N (%)	Exposed ESRD Cases N=320 N (%)	HR (95% CI)†	P for trend
FUMIGANTS					
Methyl Bromide	<490	3180 (6)	17 (5.7)	0.63 (0.38, 1.04)	
	490-1873	2449 (4.6)	20 (6.8)	0.87 (0.54, 1.40)	
	≥1874	2038 (3.8)	20 (6.8)	0.97 (0.61, 1.57)	
					0.9304
FUNGICIDES					
Benomyl	<1225	1021 (4.4)	6 (4.8)	0.89 (0.39, 2.06)	
	≥ 1225	774 (3.3)	7 (5.6)	1.18 (0.54, 2.58)	
					0.6788
Chlorothalonil*	<588	1341 (2.5)	13 (4.4)	1.47 (0.84, 2.59)	
	588-3254	1483 (2.8)	14 (4.7)	1.38 (0.80, 2.40)	
	≥3255	1330 (2.5)	14 (4.7)	1.54 (0.89, 2.67)	
					0.1119
Captan	<966	3352 (6.9)	6 (2.5)	0.45 (0.20, 1.02)	
	≥966	1006 (2.1)	5 (2.1)	0.68 (0.28, 1.66)	
					0.4214
Metalaxyl*	<294	1315 (5.6)	11 (8.7)	1.62 (0.86, 3.06)	
	294-1679	1531 (6.6)	12 (9.5)	1.58 (0.83, 3.01)	
	≥ 1680	1339 (5.7)	13 (10.3)	1.92 (1.01, 3.66)	
					0.067
HERBICIDES					
Phenoxy herbicides					
2,4-D*	<1721	15671 (29.8)	67 (22.9)	0.74 (0.54, 1.02)	
	1721-6614	12980 (24.7)	66 (22.5)	0.87 (0.62, 1.2)	
	≥ 6615	10788 (20.5)	71 (24.2)	1.00 (0.73, 1.39)	
					0.3247
2,4,5,T*	<780	2425 (10.3)	12 (9.4)	0.60 (0.33, 1.09)	
	≥ 780	1776 (7.6)	13 (10.2)	0.83 (0.46, 1.48)	
					0.5508

Table 3.4 continued

		Exposed Non-cases N=55,260	Exposed ESRD Cases N=320		
	Intensity-weighted lifetime-days	N (%)	N (%)	HR (95% CI)†	P for trend
Triazine herbicides					
Atrazine	<1302	13386 (25.3)	67 (22.3)	1.10 (0.80, 1.52)	
	1302-6439	14134 (26.7)	67 (22.3)	1.00 (0.73, 1.39)	
	≥ 6440	9065 (17.1)	72 (24.0)	1.51 (1.11, 2.06)	
					0.023
Cyanazine	<780	7791 (15.7)	27 (10.6)	0.82 (0.54, 1.26)	
	780-2787	6479 (13.1)	29 (11.4)	1.07 (0.71, 1.62)	
	≥ 2788	6201 (12.5)	28 (11)	1.16 (0.76, 1.77)	
					0.3698
Metribuzin *	<455	3555 (15.1)	14 (10.9)	0.90 (0.50, 1.62)	
	455-1322	2497 (10.6)	15 (11.6)	1.39 (0.79, 2.45)	
	≥ 1323	2627 (11.2)	16 (12.4)	1.49 (0.86, 2.59)	
					0.1075
Dinitroaniline herbicides					
Pendimethalin*	<793	4318 (18.3)	14 (11.0)	0.7 (0.4, 1.23)	
	793-3023	2829 (12)	14 (11.0)	1.19 (0.67, 2.10)	
	≥ 3024	1779 (7.6)	15 (11.8)	2.15 (1.23, 3.77)	
					0.0041
Trifluralin	<1008	8255 (16.8)	37 (14.7)	0.95 (0.66, 1.38)	
	1008-3417	8060 (16.4)	39 (15.5)	1.10 (0.76, 1.59)	
	≥ 3418	9425 (19.1)	38 (15.1)	0.91 (0.63, 1.31)	
					0.6499
Chloroacetanilide herbicides					
Metolachlor	<1006	9206 (18.7)	38 (14.5)	1.02 (0.71, 1.46)	
	1006-3827	7157 (14.5)	38 (14.5)	1.39 (0.96, 2.00)	
	≥ 3828	6443 (13.1)	40 (15.3)	1.53 (1.08, 2.18)	
					0.0121
Alachlor	<1008	9683 (19.7)	46 (17.6)	1.04 (0.74, 1.48)	
	1008-5486	9776 (19.9)	47 (18)	1.02 (0.73, 1.44)	
	≥ 5487	6111 (12.4)	49 (18.8)	1.56 (1.12, 2.18)	
					0.0077

Table 3.4 continued

		Exposed Non-cases N=55,260	Exposed ESRD Cases N=320		
	Intensity-weighted lifetime-days	N (%)	N (%)	HR (95% CI)†	P for trend
all other herbicides					
Dicamba	<473	6981 (14.2)	32 (12.4)	1.01 (0.67, 1.51)	
	473-2603	9899 (20.2)	29 (11.2)	0.66 (0.43, 1.01)	
	≥ 2604	7766 (15.8)	34 (13.1)	1.05 (0.71, 1.57)	
					0.7251
Chlorimuron-ethyl*	<351	3368 (14.3)	12 (9.3)	0.90 (0.49, 1.65)	
	351-787	1361 (5.8)	14 (10.9)	2.47 (1.4, 4.34)	
	≥ 788	2863 (12.2)	13 (10.1)	1.06 (0.59, 1.90)	
					0.5893
EPTC	<15	4525 (9.3)	11 (4.4)	0.64 (0.35, 1.19)	
	15-55	2753 (5.6)	12 (4.8)	1.18 (0.65, 2.13)	
	>55	2713 (5.6)	12 (4.8)	1.25 (0.69, 2.24)	
					0.3894
Paraquat*	<638	1881 (8)	11 (8.5)	1.15 (0.61, 2.15)	
	638-2087	1034 (4.4)	10 (7.7)	1.82 (0.93, 3.58)	
	≥ 2088	1012 (4.3)	12 (9.2)	2.23 (1.18, 4.21)	
					0.0121
Petroleum Oil*	<784	1950 (8.3)	11 (8.5)	1.27 (0.68, 2.38)	
	784-2024	1006 (4.3)	12 (9.3)	3.20 (1.75, 5.85)	
	≥ 2025	1969 (8.4)	12 (9.3)	1.42 (0.78, 2.59)	
					0.1906
Imazethapyr	<350	6748 (13.8)	29 (11.2)	1.34 (0.87, 2.07)	
	350-839	4819 (9.8)	29 (11.2)	2.03 (1.31, 3.15)	
	≥ 840	9393 (19.2)	31 (12)	1.26 (0.83, 1.93)	
					0.3169
Glyphosate	<600	12104 (22.9)	70 (23.4)	0.91 (0.67, 1.26)	
	600-2687	14471 (27.3)	72 (24.1)	0.75 (0.54, 1.03)	
	≥ 2688	13375 (25.3)	74 (24.7)	0.85 (0.62, 1.17)	
					0.5843
Butylate*	<1006	3237 (13.8)	13 (10.2)	0.78 (0.43, 1.41)	
	≥ 1006	2888 (12.3)	12 (9.4)	0.87 (0.48, 1.60)	
					0.6548

Table 3.4 continued

		Exposed Non-cases N=55,260	Exposed ESRD Cases N=320		
	Intensity-weighted lifetime-days	N (%)	N (%)	HR (95% CI)†	P for trend
INSECTICIDES					
Organochlorines					
Aldrin*	<327	1292 (5.5)	12 (9.8)	1.27 (0.68, 2.37)	
	327-1018	1196 (5.1)	11 (9)	1.21 (0.63, 2.31)	
	≥ 1019	1227 (5.2)	12 (9.8)	1.23 (0.66, 2.29)	
					0.5243
Chlordane*	<560	2581 (11)	11 (8.7)	0.57 (0.30, 1.06)	
	560-1224	805 (3.4)	10 (7.9)	1.44 (0.75, 2.78)	
	≥ 1225	874 (3.7)	13 (10.2)	1.70 (0.95, 3.06)	
					0.0409
DDT*	<438	1904 (8.2)	16 (12.7)	0.80 (0.46, 1.38)	
	438-2327	1689 (7.2)	16 (12.7)	0.82 (0.47, 1.42)	
	≥ 2328	1328 (5.7)	15 (11.9)	0.99 (0.56, 1.74)	
					0.9263
Heptachlor*	>408	1235 (5.2)	11 (8.7)	1.29 (0.68, 2.46)	
	≥ 408	1433 (6.1)	15 (11.9)	1.44 (0.82, 2.52)	
					0.2068
Toxaphene*	<1006	1554 (6.6)	8 (6.5)	0.69 (0.34, 1.42)	
	≥ 1006	975 (4.1)	9 (7.3)	0.99 (0.49, 1.99)	
					0.9583
Organophosphates					
Terbufos	<827	7124 (14.4)	28 (11)	1.0 (0.66, 1.51)	
	827-2159	4413 (8.9)	27 (10.6)	1.41 (0.93, 2.13)	
	≥ 2160	6833 (13.8)	29 (11.4)	0.97 (0.65, 1.44)	
					0.9823
Fonofos	<588	3761 (7.6)	10 (3.9)	0.62 (0.33, 1.19)	
	588-1619	2834 (5.7)	11 (4.3)	0.9 (0.48, 1.66)	
	≥ 1620	3670 (7.4)	12 (4.7)	0.7 (0.39, 1.27)	
					0.257
Chlorpyrifos	<438	5989 (13.1)	29 (11)	0.85 (0.57, 1.26)	
	438-2139	7603 (16.7)	28 (10.6)	0.65 (0.43, 0.97)	
	≥ 2140	6078 (13.3)	29 (11)	0.84 (0.57, 1.25)	
					0.3738

Table 3.4 continued

		Exposed Non-cases N=55,260	Exposed ESRD Cases N=320		
	Intensity-weighted lifetime-days	N (%)	N (%)	HR (95% CI)†	P for trend
Malathion*	<644	6577 (28.2)	27 (21.4)	0.87 (0.54, 1.42)	
	644-1743	3567 (15.3)	28 (22.2)	1.47 (0.91, 2.36)	
	≥ 1744	4403 (18.9)	28 (22.2)	1.01 (0.62, 1.63)	
					0.9247
Parathion*	<1392	1022 (4.4)	8 (6.5)	1.11 (0.53, 2.29)	
	≥1392	687 (2.9)	8 (6.5)	1.64 (0.79, 3.43)	
					0.1856
Diazinon*	<1184	3124 (13.4)	11 (8.7)	0.57 (0.31, 1.07)	
	≥1184	1762 (7.5)	14 (11.1)	1.11 (0.62, 1.97)	
					0.6733
Phorate*	<408	2514 (10.7)	10 (8.3)	0.76 (0.39, 1.51)	
	408-2169	2625 (11.2)	12 (9.9)	0.87 (0.47, 1.63)	
	≥ 2170	1486 (6.3)	12 (9.9)	1.47 (0.80, 2.71)	
					0.1941
Coumaphos	<957	2100 (4.3)	14 (5.6)	1.29 (0.75, 2.22)	
	≥957	1651 (3.4)	14 (5.6)	1.63 (0.95, 2.79)	
					0.0689
Dichlorvos	<3136	2987 (6.1)	10 (4.1)	0.78 (0.41, 1.47)	
	≥ 3136	1682 (3.4)	10 (4.1)	1.41 (0.74, 2.67)	
					0.2862
Pyrethroids					
Permethrin for crops	<368	2737 (5.6)	11 (4.3)	1.06 (0.58, 1.94)	
	368-2572	2611 (5.3)	11 (4.3)	0.99 (0.54, 1.82)	
	≥ 2573	1576 (3.2)	11 (4.3)	1.50 (0.82, 2.77)	
					0.201
Permethrin for animals	<646	2709 (5.4)	13 (5.1)	1.60 (0.91, 2.83)	
	≥ 646	3225 (6.5)	12 (4.7)	1.24 (0.69, 2.22)	
					0.4335

Table 3.4 continued

		Exposed Non-cases N=55,260	Exposed ESRD Cases N=320		
	Intensity-weighted lifetime-days	N (%)	N (%)	HR (95% CI)†	P for trend
Carbamates					
Carbofuran	<696	5740 (11.7)	21 (8.4)	0.65 (0.41, 1.03)	
	696-2299	3753 (7.6)	21 (8.4)	0.88 (0.56, 1.39)	
	≥ 2300	3210 (6.5)	22 (8.8)	1.06 (0.68, 1.65)	
					0.755
Carbaryl*	<919	4818 (20.6)	18 (14.4)	0.57 (0.34, 0.97)	
	919-6874	3353 (14.4)	17 (13.6)	0.59 (0.33, 1.05)	
	≥ 6875	1649 (7.1)	19 (15.2)	1.05 (0.58, 1.88)	
					0.3186
Aldicarb*	<1323	878 (3.7)	8 (6.3)	1.66 (0.79, 3.50)	
	≥ 1323	856 (3.6)	7 (5.5)	1.71 (0.77, 3.79)	
					0.1876

†Referent group = pesticide applicators who reported that they did not use the specific pesticide being analyzed.
* Indicates pesticide with duration and frequency information only available on the take-home questionnaire: N (non-cases) = 24,429 and N (cases) =136.

Table 3.5: Sensitivity analysis for Aim 1 - Intensity-weighted lifetime-days of use of specific pesticides and ESRD risk, adjusted for age and state, among male pesticide applicators in the Agricultural Health Study (1993-1997), excluding: cases diagnosed within 5 years after enrollment and 5 years of post-enrollment person-time for non-cases

Pesticides	Intensity-weighted lifetime-days	**ESRD Cases N (%) (N=268)**	**HR (95% CI)**	p-value
FUMIGANTS				
Methyl Bromide	<490	16 (6.4)	0.69 (0.41, 1.18)	
	490-1873	17 (6.8)	0.86 (0.51, 1.44)	
	≥1874	17 (6.8)	0.97 (0.58, 1.62)	
				0.9992
FUNGICIDES				
Chlorothalonil *	<588	13 (5.2)	1.75 (0.99, 3.08)	
	588-3254	13 (5.2)	1.51 (0.85, 2.67)	
	≥3255	10 (4)	1.29 (0.67, 2.46)	
				0.3986
Metalaxyl*	<294	9 (8.5)	1.65 (0.82, 3.31)	
	294-1679	10 (9.4)	1.71 (0.84, 3.49)	
	≥ 1680	13 (12.3)	2.53 (1.29, 4.96)	
				0.0113
HERBICIDES				
Phenoxy herbicides				
2,4-D*	<1721	59 (23.6)	0.79 (0.56, 1.12)	
	1721-6614	56 (22.4)	0.89 (0.62, 1.28)	
	≥ 6615	61 (24.4)	1.03 (0.73, 1.47)	
				0.3647
2,4,5-T*	<780	9 (8.4)	0.52 (0.26, 1.04)	
	≥ 780	11 (10.3)	0.82 (0.44, 1.55)	
				0.5749
Triazine herbicides				
Atrazine	<1302	59 (23.1)	1.1 (0.79, 1.55)	
	1302-6439	56 (22)	0.95 (0.67, 1.35)	
	≥ 6440	56 (22)	1.3 (0.92, 1.83)	
				0.145
Cyanazine	<780	25 (11.5)	0.9 (0.57, 1.41)	
	780-2787	23 (10.6)	0.95 (0.6, 1.52)	
	≥ 2788	22 (10.1)	1.07 (0.67, 1.71)	
				0.7125
Metribuzin *	<455	12 (11.1)	0.92 (0.48, 1.74)	
	455-1322	13 (12)	1.45 (0.79, 2.67)	
	≥ 1323	14 (13)	1.57 (0.87, 2.84)	
				0.0958

Table 3.5 continued

Pesticides	Intensity-weighted lifetime-days	**ESRD Cases N (%) (N=268)**	**HR (95% CI)**	p-value
Dinitroaniline herbicides				
Pendimethalin *	<793	12 (11.2)	0.7 (0.38, 1.3)	
	793-3023	13 (12.1)	1.3 (0.72, 2.36)	
	≥ 3024	12 (11.2)	2.04 (1.09, 3.81)	
				0.0144
Trifluralin	<1008	33 (15.6)	1.03 (0.69, 1.53)	
	1008-3417	32 (15.1)	1.1 (0.73, 1.64)	
	≥ 3418	32 (15.1)	0.93 (0.62, 1.39)	
				0.696
Chloroacetanilide herbicides				
Metolachlor	<1006	35 (15.8)	1.08 (0.74, 1.59)	
	1006-3827	27 (12.2)	1.14 (0.74, 1.73)	
	≥ 3828	33 (14.9)	1.45 (0.99, 2.14)	
				0.0597
Alachlor	<1008	41 (18.5)	1.1 (0.76, 1.6)	
	1008-5486	42 (18.9)	1.08 (0.75, 1.55)	
	≥ 5487	38 (17.1)	1.42 (0.98, 2.07)	
				0.0737
all other herbicides				
Dicamba	<473	31 (14)	1.25 (0.82, 1.91)	
	473-2603	26 (11.8)	0.76 (0.48, 1.2)	
	≥ 2604	27 (12.2)	1.07 (0.69, 1.67)	
				0.8944
Chlorimuron-ethyl*	<351	10 (9.2)	0.87 (0.45, 1.7)	
	351-787	11 (10.1)	2.28 (1.21, 4.31)	
	≥ 788	13 (11.9)	1.28 (0.71, 2.31)	
				0.2826
EPTC	<15	11 (5.1)	0.78 (0.42, 1.45)	
	15-55	11 (5.1)	1.32 (0.71, 2.45)	
	>55	9 (4.2)	1.14 (0.58, 2.23)	
				0.5962
Paraquat*	<638	7 (6.4)	0.86 (0.4, 1.88)	
	638-2087	9 (8.2)	2 (0.98, 4.11)	
	≥ 2088	11 (10)	2.5 (1.28, 4.89)	
				0.0055
Petroleum Oil *	<784	8 (7.3)	1.09 (0.53, 2.26)	
	784-2024	11 (10.1)	3.5 (1.86, 6.6)	
	≥ 2025	11 (10.1)	1.54 (0.82, 2.89)	
				0.1278
Imazethapyr	<350	22 (10)	1.15 (0.71, 1.87)	
	350-839	26 (11.9)	2.07 (1.3, 3.29)	
	≥ 840	23 (10.5)	1.01 (0.62, 1.64)	
				0.9064

Table 3.5 continued

Pesticides	Intensity-weighted lifetime-days	**ESRD Cases N (%) (N=268)**	**HR (95% CI)**	p-value
Glyphosate	<600	60 (23.7)	0.92 (0.65, 1.3)	
	600-2687	58 (22.9)	0.69 (0.49, 0.99)	
	≥ 2688	65 (25.7)	0.87 (0.62, 1.22)	
				0.7717
Butylate *	<1006	10 (9.3)	0.7 (0.36, 1.36)	
	≥ 1006	12 (11.2)	1.03 (0.56, 1.89)	
				0.9244
INSECTICIDES				
Organochlorines				
Aldrin *	<327	10 (9.8)	1.21 (0.61, 2.40)	
	327-1018	9 (8.8)	1.13 (0.55, 2.30)	
	≥ 1019	10 (9.8)	1.16 (0.59, 2.30)	
				0.6787
Chlordane*	<560	10 (9.3)	0.63 (0.33, 1.23)	
	560-1224	9 (8.4)	1.61 (0.80, 3.22)	
	≥ 1225	12 (11.2)	1.99 (1.07, 3.68)	
				0.0136
DDT*	<438	12 (11.3)	0.71 (0.38, 1.33)	
	438-2327	14 (13.2)	0.86 (0.48, 1.55)	
	≥ 2328	13 (12.3)	1.03 (0.56, 1.89)	
				0.7826
Heptachlor*	>408	7 (6.6)	0.91 (0.41, 2.01)	
	≥ 408	13 (12.3)	1.37 (0.75, 2.51)	
				0.3071
Toxaphene*	<1006	8 (7.6)	0.82 (0.40, 1.69)	
	≥ 1006	7 (6.7)	0.93 (0.42, 2.04)	
				0.8395
Organophosphates				
Terbufos	<827	25 (11.6)	1.04 (0.67, 1.62)	
	827-2159	22 (10.2)	1.39 (0.88, 2.2)	
	≥ 2160	28 (13)	1.12 (0.75, 1.69)	
				0.5144
Fonofos	<588	7 (3.2)	0.5 (0.23, 1.08)	
	588-1619	6 (2.8)	0.56 (0.25, 1.28)	
	≥ 1620	10 (4.6)	0.61 (0.31, 1.2)	
				0.1245
Chlorpyrifos	<438	23 (10.4)	0.79 (0.51, 1.23)	
	438-2139	24 (10.8)	0.66 (0.42, 1.01)	
	≥ 2140	26 (11.7)	0.88 (0.58, 1.34)	
				0.5742

Table 3.5 continued

Pesticides	Intensity-weighted lifetime-days	**ESRD Cases N (%) (N=268)**	**HR (95% CI)**	p-value
Malathion *	<644	23 (21.7)	0.9 (0.53, 1.53)	
	644-1743	24 (22.6)	1.53 (0.91, 2.58)	
	≥ 1744	24 (22.6)	1.08 (0.64, 1.83)	
				0.7319
Parathion *	<1392	8 (7.7)	1.37 (0.66, 2.86)	
	≥1392	6 (5.8)	1.53 (0.66, 3.57)	
				0.3265
Diazinon*	<1184	9 (8.5)	0.57 (0.28, 1.13)	
	≥1184	13 (12.3)	1.28 (0.70, 2.34)	
		()		0.3785
Phorate*	<408	9 (8.9)	0.75 (0.37, 1.53)	
	408-2169	7 (6.9)	0.56 (0.25, 1.23)	
	≥ 2170	10 (9.9)	1.38 (0.71, 2.69)	
				0.3661
Coumaphos	<957	12 (5.6)	1.32 (0.74, 2.37)	
	≥957	13 (6.1)	1.81 (1.03, 3.17)	
				0.0345
Dichlorvos	<3136	9 (4.3)	0.84 (0.43, 1.65)	
	≥ 3136	10 (4.8)	1.7 (0.89, 3.23)	
				0.1051
Pyrethroids				
Permethrin for crops	<368	8 (3.7)	0.90 (0.44, 1.83)	
	368-2572	10 (4.7)	1.05 (0.55, 1.99)	
	≥ 2573	10 (4.7)	1.57 (0.83, 2.99)	
				0.1666
Permethrin for animals	<646	9 (4.2)	1.29 (0.65, 2.53)	
	≥ 646	10 (4.7)	1.2 (0.63, 2.28)	
				0.5607
Carbamates				
Carbofuran	<696	17 (8)	0.62 (0.38, 1.03)	
	696-2299	16 (7.5)	0.79 (0.47, 1.33)	
	≥ 2300	20 (9.4)	1.12 (0.7, 1.79)	
				0.614
Carbaryl *	<919	16 (15.2)	0.61 (0.35, 1.07)	
	919-6874	14 (13.3)	0.61 (0.32, 1.15)	
	≥ 6875	15 (14.3)	1.06 (0.55, 2.05)	
				0.3847
Aldicarb *	<1323	8 (7.4)	2.04 (0.96, 4.36)	
	≥ 1323	5 (4.6)	1.50 (0.59, 3.81)	
				0.3996

* Indicates pesticides with duration and frequency information only available on the take-home questionnaire: N (non-cases) = 23,812 and N (cases) =114.

Table 3.6: Intensity-weighted lifetime-days of use of specific chemicals by pesticide applicators, stratified by state

		Iowa						North Carolina					
		Non-cases		Cases				Non-cases		Cases			
Pesticide		**N**	**%**	**N**	**%**	**HR**	**95% CI**	**N**	**%**	**N**	**%**	**HR**	**95% CI**
2,4-D	None	6185	17.7	21	16.4	.	.	6949	39.4	68	41.2	.	.
	<1721	11131	31.8	32	25	0.76	0.44, 1.32	4540	25.8	35	21.2	0.72	0.48, 1.09
	1721-6614	9871	28.2	33	25.8	0.73	0.42, 1.26	3109	17.6	33	20	1.02	0.67, 1.54
	≥ 6615	7763	22.2	42	32.8	1.01	0.60, 1.71	3025	17.2	29	17.6	0.93	0.6, 1.43
2,4,5-T	None	13228	81	58	81.7	.	.	6025	84.7	44	78.6	.	.
	<780	1879	11.5	7	9.9	0.52	0.24, 1.12	546	7.7	5	8.9	0.89	0.36, 2.19
	≥ 780	1231	7.5	6	8.5	0.66	0.29, 1.51	545	7.7	7	12.5	1.21	0.55, 2.65
Atrazine	None	8324	23.7	27	20.3	.	.	8008	45.1	67	40.1	.	.
	<1302	10211	29	38	28.6	1.04	0.64, 1.71	3175	17.9	29	17.4	1.11	0.72, 1.72
	1302-6439	10643	30.3	42	31.6	1.03	0.64, 1.68	3491	19.7	25	15	0.88	0.55, 1.39
	≥ 6440	5994	17	26	19.5	1.10	0.64, 1.88	3071	17.3	46	27.5	1.82	1.25, 2.65
Metolachlor	None	16093	47.4	53	43.4	.	.	10455	67.7	93	66.4	.	.
	<1006	7488	22.1	27	22.1	1.14	0.72, 1.81	1718	11.1	11	7.9	0.84	0.45, 1.56
	1006-3827	5695	16.8	24	19.7	1.42	0.87, 2.29	1462	9.5	14	10	1.39	0.8, 2.44
	≥ 3828	4646	13.7	18	14.8	1.39	0.81, 2.37	1797	11.6	22	15.7	1.73	1.08, 2.75
Alachlor	None	15062	44.7	48	40	.	.	8460	55.1	71	50.4	.	.
	<1008	7767	23	33	27.5	1.09	0.7, 1.7	1916	12.5	13	9.2	0.87	0.48, 1.56
	1008-5486	7280	21.6	24	20	0.87	0.53, 1.42	2496	16.2	23	16.3	1.2	0.75, 1.92
	≥ 5487	3620	10.7	15	12.5	1.18	0.66, 2.1	2491	16.2	34	24.1	1.82	1.21, 2.74
Glyphosate	None	9987	28.4	43	32.3	.	.	2996	16.9	40	24.1	.	.
	<600	8556	24.3	32	24.1	0.94	0.59, 1.48	3548	20	38	22.9	0.88	0.56, 1.37
	600-2687	9204	26.2	28	21.1	0.82	0.51, 1.32	5267	29.7	44	26.5	0.68	0.44, 1.05
	≥ 2688	7443	21.2	30	22.6	1.16	0.73, 1.86	5932	33.4	44	26.5	0.67	0.44, 1.03

Table 3.6 continued

		Iowa						North Carolina					
		Non-cases		Cases				Non-cases		Cases			
Pesticide		**N**	**%**	**N**	**%**	**HR**	**95% CI**	**N**	**%**	**N**	**%**	**HR**	**95% CI**
Trifluralin	None	13070	38.3	46	38	.	.	10473	69	91	70	.	.
	<1008	6622	19.4	23	19	0.92	0.56, 1.52	1633	10.8	14	10.8	1.01	0.58, 1.77
	1008-3417	6810	20	28	23.1	1.11	0.7, 1.79	1250	8.2	11	8.5	1.05	0.57, 1.95
	≥ 3418	7592	22.3	24	19.8	0.84	0.51, 1.38	1833	12.1	14	10.8	1.04	0.59, 1.82
Terbufos	None	19340	56.7	63	52.1	.	.	11711	76.5	107	80.5	.	.
	<827	6198	18.2	22	18.2	1.11	0.68, 1.8	926	6	6	4.5	0.87	0.39, 1.94
	827-2159	3598	10.5	19	15.7	1.52	0.91, 2.53	815	5.3	8	6	1.33	0.66, 2.70
	≥ 2160	4973	14.6	17	14	1.00	0.58, 1.7	1860	12.1	12	9	0.96	0.53, 1.74
Chlorpyrifos	None	17186	56	84	67.7	.	.	8744	58.6	93	66.9	.	.
	<438	4408	14.4	16	12.9	0.81	0.47, 1.37	1581	10.6	13	9.4	0.93	0.52, 1.66
	438-2139	5423	17.7	13	10.5	0.53	0.3, 0.94	2180	14.6	15	10.8	0.82	0.48, 1.42
	≥ 2140	3670	12	11	8.9	0.69	0.37, 1.29	2408	16.1	18	12.9	1.00	0.6, 1.67
Malathion	None	5874	36.2	27	38.6	.	.	2863	40.6	16	28.6	.	.
	<644	5296	32.6	18	25.7	0.73	0.4, 1.34	1281	18.2	9	16.1	1.18	0.52, 2.66
	644-1743	2597	16	16	22.9	1.27	0.68, 2.36	970	13.7	12	21.4	1.86	0.88, 3.92
	≥ 1744	2462	15.2	9	12.9	0.75	0.35, 1.58	1941	27.5	19	33.9	1.36	0.7, 2.66
Coumaphos	None	30775	92.1	104	88.9	.	.	14069	92.7	120	88.9	.	.
	<957	1562	4.7	8	6.8	1.26	0.62, 2.55	538	3.5	6	4.4	1.47	0.67, 3.25
	≥957	1073	3.2	5	4.3	1.39	0.59, 3.3	578	3.8	9	6.7	1.99	1.02, 3.86
Carbofuran	None	25252	75	87	74.4	.	.	11116	72.2	100	74.6	.	.
	<696	4425	13.1	15	12.8	0.74	0.43, 1.28	1315	8.5	6	4.5	0.53	0.24, 1.18
	696-2299	2438	7.2	8	6.8	0.72	0.35, 1.46	1315	8.5	13	9.7	1.08	0.61, 1.91
	≥ 2300	1568	4.7	7	6	0.99	0.47, 2.11	1642	10.7	15	11.2	1.13	0.66, 1.94
Carbaryl	None	11416	69.9	54	76.1	.	.	2103	30	17	31.5	.	.
	<919	3519	21.6	10	14.1	0.53	0.27, 1.04	1299	18.5	8	14.8	0.68	0.3, 1.58
	919-6874	1170	7.2	6	8.5	0.96	0.42, 2.19	2183	31.1	11	20.4	0.49	0.23, 1.05
	≥ 6875	221	1.4	1	1.4	1.05	0.2, 5.46	1428	20.4	18	33.3	1.05	0.54, 2.04

CHAPTER 4: Environmental and Occupational Pesticide Exposure and End Stage Renal Disease Risk among Farmers' Wives in the Agricultural Health Study

Introduction

Women who live on farms, in areas where pesticides are regularly applied, are likely to have higher and more varied pesticide exposures than the general population. They may be exposed to pesticides through pesticide-contaminated drinking water, spray drift, or by handling items that have been contaminated through pesticide application activities. Proximity of household to pesticide application areas has been positively correlated with levels of pesticides found in household dust (23, 35), and several large drinking water surveys have found widespread contamination of community water systems and domestic wells by pesticides and pesticide degradates (36-39). Pesticide residues tracked into the home and vehicles from pesticides on applicators' skin, shoes, and clothing (referred to as 'take-home' exposures) represent another important route of exposure for family members who do not engage in agricultural work (23, 24, 124, 147).Yet another route of exposure common to women on and off farms is through home and lawn pest management: chlorophenoxy herbicides, and carbamate, pyrethroid, and organophosphate insecticides are common in home and lawn care (40), several of which are considered to be moderately to highly toxic depending on the formulation and concentration (41). Finally, farm wives may be exposed occupationally, through mixing and applying agricultural pesticides or working in pesticide-treated fields (148).

Experimental animal studies and poisoning case studies suggest that pesticide exposure may cause permanent kidney damage, but epidemiological research on the effects of prolonged low-dose exposure on the human renal system is limited. In accompanying analyses, we reported that long-term use of several specific pesticides was associated with end-stage renal disease (ESRD) among a large cohort of

pesticide applicators. The wives of pesticide applicators are likely to have different patterns of pesticide exposure from their husbands, including less frequent use of pesticides and use of less toxic pesticides (148), and certain indirect exposures such as may occur via washing of pesticide-contaminated clothing. The Agricultural Health Study (AHS) provides a unique opportunity to study the effects of a variety of exposure pathways among a large population of farmers' wives. Using AHS data, we examined ESRD risk among wives of farmers in relation to use of individual pesticides by wives and by their applicator husbands. We also evaluated the association between other non-application pesticide exposure opportunities and risk of ESRD.

Methods

Study population and case definition:

The AHS is a large, prospective study of Iowa and North Carolina pesticide applicators and their spouses (26). Approximately 80% (N=52,394) of licensed private applicators (mostly farmers) in Iowa and North Carolina enrolled in the AHS by completing a questionnaire when they received or renewed their pesticide training certification. A total of 32,347 spouses (75% of those eligible) enrolled in the study by completing a self-administered questionnaire (81%) or a telephone interview (19%). Enrollment questionnaires collected information on demographics, medical conditions, medication use, lifestyle factors, and pesticide use. Of the enrolled applicators, 44% also completed a take-home questionnaire, which collected additional information on specific pesticide use and pesticide application practices. Because the distribution of ESRD risk factors differ by gender, and because <1% of all spouses were male, the current analysis includes only female spouses of pesticide applicators. We also excluded spouses under age 18 (N=4) and those who were diagnosed with end-stage renal disease prior to enrollment (N=25; 20% of female spouses with diagnosed ESRD), leaving 32,099 wives for analysis (Figure 3.1). Through a linkage with the USRDS, we ascertained diagnosis dates for ESRD cases diagnosed between study enrollment and end of follow-up (December 31, 2011). Date of death was obtained by linking the cohort to state mortality files and the National Death Index.

Exposure Assessment

Pesticide exposure information from the spouse enrollment questionnaire included: 1) ever/never use of 50 specific pesticides; 2) number of years (duration) and days per year (frequency) personally mixed or applied pesticides in general; 3) number of years lived or worked on a farm; 4) specific farm tasks performed; 5) performance of household tasks involving possible pesticide exposure; 6) distance from the participant's house to fields where pesticides were applied; 7) household practices that could increase pesticide exposure (eg. storage of pesticides in the home); and 8) treatment of the home or lawn for pests. The applicator enrollment questionnaire elicited information on ever use of 50 pesticides and duration and frequency of use for 22 of those pesticides. On the applicator take-home questionnaire, applicators provided information on duration and frequency of use of the remaining 28 pesticides, as well as distance from private well to the nearest pesticide application area. (The questionnaires may be viewed at: http://aghealth.nih.gov/collaboration/questionnaires.html.)

Direct exposure was defined as the wives' ever personal use of 50 specific pesticides and general pesticide use. The husbands' ever and cumulative use of specific chemicals was used to approximate wives' *indirect exposure*. Additionally, we evaluated ESRD risk in relation to several *residential pesticide exposures,* including household pesticide use, washing pesticide-exposed clothing with the family wash, pesticide-exposed work boots left on in the home, storage of pesticides in the home, distance between home and pesticide application area, and distance between private well and pesticide mixing area. Pesticide exposure may occur through contact with crops after a recent pesticide application and spending time outdoors during pesticide application (149), and the opportunity for exposure is greater for those who have lived and/or worked on a farm for their whole lives compared to those who have spent less time on the farm. Therefore, we also considered the number of days spent working in the fields during the growing season prior to enrollment, the number of hours per day spent in the sun during the growing season, the number of years spent living or working on a farm over the lifetime, ever having a non-farm job, and specific farm work activities (other than pesticide application) as potential risk factors. This last cluster of potential exposures will be referred to as '*non-application farming exposures'*.

Statistical analyses

We used Cox proportional hazards models with age as the time scale to evaluate associations between ESRD risk and potential pesticide exposures. Participants accrued person-time from the date of enrollment to the earliest of ESRD diagnosis, death, or end of study follow-up.

Analyses of direct exposures, including duration, frequency, and cumulative use of pesticides in general, and ever use of specific pesticides, were limited to women who had ever personally mixed or applied pesticides (N=17,425). Cumulative use of pesticides in general (i.e. the product of duration and frequency of use) was categorized into quartiles.

Husbands' ever and cumulative use of specific chemicals (i.e. indirect exposure) was evaluated among wives who reported no agricultural pesticide use (N=13,717). Exposure-response analyses of husbands' cumulative use of specific chemicals accounted for the estimated amount of time that wives lived with their husbands prior to enrollment. We obtained information collected during AHS Phase 3 (2010-2012: N (applicators) = 24,171 and N (spouses) = 19,959) on the number of years that married participants reported living together prior to enrollment. For those who did not participate in Phase 3 or for whom this information was missing, we imputed values based on the age-specific average number of years that Phase 3 wives reported living with their husbands before enrollment. This allowed us to estimate a date that couples began living together. The date of first use for each chemical was the midpoint of the husbands' reported decade of first use. On average, 6% of pesticide users did not report decade of first use. In this case, the date of first use was the enrollment date minus the number of years of use, or for pesticides banned prior to enrollment, the midpoint of the ban year + 5 years, minus the number of years of use. The wives' pesticide-specific exposure duration was then defined as the number of years that wives could be exposed based on the estimated start date for living together, the estimated date that husbands initiated use of a specific pesticide, and the number of years that husbands reported using that pesticide. As such, a husband's use of a specific pesticide prior to living with his wife would not count towards his wife's duration of exposure. For exposure-response analyses, we multiplied the wives' pesticide exposure duration by the husbands' frequency of use to obtain estimated lifetime-days of

indirect exposure to specific chemicals, and then categorized lifetime-days into three levels: none, ≤ non-zero median lifetime-days, > median lifetime-days.

Initially, we evaluated ESRD risk in relation to residential and non-application farming exposures among all wives. To explore potential risk patterns among farm wives who did not themselves use pesticides, we also restricted these analyses to wives who reported no agricultural pesticide use. Figure 3.1 depicts the number of wives in each analysis. Exposure-response trends for variables with more than two levels were assessed with linear trend tests, using the median value or the midpoint value of each category as the exposure value, as appropriate. We present hazard ratio estimates only for those exposures for which there were at least three cases in each exposure stratum.

State and education were identified as potential confounders through a literature review, but were not adjusted for in the present analyses because they were not strongly associated with ESRD in our study population. Though self-reported doctor diagnosis of diabetes at enrollment, body mass index (BMI), and use of non-steroidal anti-inflammatory drugs (NSAIDs) were clearly associated with ESRD, we did not adjust for these factors because diabetes and BMI may be on the causal pathway between pesticide exposure and ESRD, and there is no evidence to suggest that use of NSAIDs would increase pesticide exposure. Wives' ever use of pesticides in general was also evaluated as a potential confounder of associations with residential and non-application farming exposures, and was found to be significantly inversely associated with ESRD risk, significantly associated with each exposure measure, and not on the causal pathway between exposure and ESRD risk. Therefore, models evaluating residential and non-application farming exposures among all wives were adjusted for wives' ever use of any pesticide, with very little loss of precision.

We used the AHS dataset release P1REL0310. All statistical analyses were done using SAS v9.3 (Cary, NC).

Results

Overall, a total of 103 cases (0.3% of wives) were diagnosed with ESRD during an average of 15.4 years of follow-up, resulting in an incidence rate of 20.8 ESRD cases per 100,000 person-years. After adjusting for age, ESRD risk was significantly higher for those who lived in North Carolina at enrollment, heavy smokers, obese participants, frequent NSAIDs users, and those who reported having a doctor-diagnosed diabetes and hypertension. (Table 4.1). ESRD risk was lower for light alcohol consumption vs. none.

Wives' direct exposure to pesticides and indirect exposure through husbands' use

Among all wives, ever use of any pesticide was inversely associated with ESRD risk (HR = 0.42; 95% CI: 0.28, 0.64: Table 4.4). However, among the sub-cohort of wives who did mix or apply pesticides, associations with ESRD risk were observed for the highest category of cumulative lifetime-days, frequency, and duration of use of any pesticides vs. the lowest category (Table 4.2). Although a trend test was significant for ESRD risk in relation to cumulative use and frequency of overall pesticide use, this finding was driven mainly by the estimates in the highest level of these measures.

Among the 17,425 women who applied pesticides, we identified 34 ESRD cases, and we had sufficient numbers to evaluate 10 specific chemicals and 6 chemical classes (Table 4.2). Among the 13,717 women who did not apply pesticides, we identified 64 ESRD cases, and there was sufficient use among their husbands to assess 43 specific chemicals for ever/never use and 23 for exposure-response analyses (Table 4.3). Data on wives' ever use of any pesticide were missing for five cases. We found a positive association between ESRD risk and ever use of chlorocetanilide herbicides (alachlor and metolachlor) for both direct (wives' use) and indirect exposures (husbands' use). Consistent with this finding, the magnitude of the estimate for the association between alachlor use and ESRD was elevated for wives' use and husbands' use, with a moderate positive trend observed for husbands' cumulative use (Table 4.5). Only 2 wives with ESRD reported use of metolachlor. ESRD risk appeared to be elevated in association with direct, but not indirect, exposure to the herbicides chlorimuron-ethyl (direct HR = 4.03;

95% CI: 1.3, 12.51; indirect HR = 1.11; 95% CI: 0.64, 1.93) and imazethapyr (HR=2.37; 95% CI: 0.76, 7.36), though only 3 cases reported using each of these pesticides.

No meaningful associations were found with the wives' use of the remaining herbicides with sufficient numbers for evaluation (atrazine, 2, 4 -D, glyphosate, petroleum oil); however, ESRD risk among non-applying wives increased with the husbands' use thiocarbamate herbicides (butylate and EPTC). This association was driven primarily by a significant positive association between ESRD and husbands' ever use of butylate (HR=1.93, 95% CI: 1.14, 3.26). We observed a corresponding positive trend with husbands' cumulative use of this chemical (p= 0.0043). Ever use of the herbicide paraquat by the husbands was also significantly associated with ESRD risk among non-applying wives overall (HR=2.33, 95% CI: 1.38, 3.95), with an observed positive exposure-response trend.

Results were mixed for insecticides. ESRD risk was elevated with husbands' use of the organophosphate insecticide dichlorvos, with a moderate positive trend observed with increasing dichlorvos use. Non-significant inverse associations were found with husbands' use of the organophosphates parathion and diazinon and the organochlorine lindane. Direct, but not indirect, exposure to the carbamate insecticide carbaryl appeared to be associated with increased ESRD risk. No clear associations or patterns were observed among the other pesticides with sufficient numbers of cases for analysis.

Residential and non-application farming exposures

After adjusting for age and any use of pesticides, we found little evidence of association between other measures of potential indirect pesticide exposure and ESRD risk among all wives (Table 4.4). ESRD risk was elevated for >10 hours spent in the sun each day during the growing season (vs. <1 hour), and we found a significant positive exposure-response trend for hours spent in the sun during the growing season 10 years before enrollment, but not during the growing season immediately prior to enrollment. No meaningful associations were observed with other farming activities, including tilling the soil, planting, applying manure and chemical fertilizer, driving combines or other crop harvesters, and hand

picking crops during the growing season immediately prior to enrollment (data not shown). ESRD risk was modestly elevated for those who reported never having a job off the farm.

Among residential exposures, risk was non-significantly elevated for participants who reported washing clothing worn during pesticide use with the family wash (compared to not washing such clothing with the family wash) 10 years prior to enrollment; however, associations with personally washing such clothing did not monotonically increase with increasing frequency of washing. Because the distance between private well and pesticide mixing activity was reported by the 44% of applicator husbands who returned the take-home questionnaire, data for this factor were missing for nearly half of wives. Having a private well on a farm where pesticides are mixed (compared to no private well or no pesticide mixing on the farm) appeared to increase the risk of ESRD, but we did not see a clear trend with increasing distance between the well and the pesticide mixing area. In general, estimates were similar but often greater in magnitude when we restricted analyses of these risk factors to spouses who reported no pesticide use, though statistical power was limited for these sub-analyses (Table 4.6).

Discussion

Among spouses who applied pesticides, ESRD risk was elevated for the highest category of duration, frequency, and lifetime use of pesticides overall, as well as for several specific pesticides, suggesting that personal pesticide use may be a risk factor for ESRD. Risk of ESRD was increased among wives whose husbands' reported ever using several specific chemicals, particularly paraquat and butylate. An apparent positive trend was observed for husbands' cumulative use of butylate, but no clear trends were observed for the 23 other chemicals for which we had sufficient numbers to conduct exposure-response analyses. We also found some evidence of an association with residential pesticide exposures, including modestly increased risks related to proximity of pesticide mixing to one's private well and washing pesticide-exposed clothing. Additionally, ESRD risk increased with increasing hours spent in the sun during the growing season, but no other factors related to farm work were associated with ESRD risk.

ESRD risk was greatest for the highest quantile of duration, and frequency, and lifetime-days of general pesticide use, among wives who reported ever mixing or applying pesticides. This pattern is consistent with the results of a previous study by this authorship team, in which we found null or non-significant associations for ESRD risk with fewer cumulative lifetime-days of use of certain chemicals by pesticide applicators, but significantly increased risk in the highest tertile of cumulative use for those same chemicals. The only other study to evaluate the association between potential pesticide exposure and ESRD risk found a significant positive association with history of occupational exposure to "frequent or daily exposure to insect or plant spray" (22); however, this estimate was unadjusted, leaving questions about potential bias related to age and other potential confounders.

Personal use of several pesticides by wives appeared to increase the risk of ESRD, but only alachlor showed a consistently positive relationship with risk across analyses of direct and indirect exposures. Though neither of these associations were statistically significant, our finding of moderately increased risk with alachlor use is similar to that observed in our previous analysis of applicators (HR for highest tertile of cumulative use vs. none: 1.56; 95% CI: 1.12, 2.18). According to a report published by the California EPA (150), rats exposed to alachlor developed chronic nephritis and increased absolute kidney weights, but we were unable to find any additional studies to confirm these results. Though we saw an increased risk with personal use of chlorimuron ethyl, the estimate was very imprecise, and this clear positive association was not reflected in the husbands' use.

Our finding of increased risk with the husbands' use of butylate contrasts with results from our previous analysis, which did not indicate an association between butylate use and ESRD risk among pesticide applicators (Lebov et al 2014). However, an experimental study in mice observed kidney lesions following administration of high doses of butylate (151). To our knowledge, no other experimental studies have evaluated the renal effects of butylate, but, among AHS women, butylate use was significantly associated with gestational diabetes (152), which is a risk factor for kidney disease (153).

Wives whose husbands' reported ever using paraquat had more than double the risk of being diagnosed with ESRD compared to wives whose husbands did not use paraquat. Acute exposures to

paraquat have been found to cause kidney damage in humans (17, 46, 154); however, little is known about the effects of chronic low-level exposure to this chemical. In our previous study, we observed a significant positive trend in ESRD risk in relation to increasing lifetime exposure to paraquat among pesticide applicators. Exposure to paraquat and other pesticides produces reactive oxygen species and related oxidative stress in renal cells (155), which can lead to renal cell apoptosis and necrosis (156); therefore, one possible biological mechanism for the association with paraquat and other oxidative stressors is through repeated exposures over the lifetime, causing slow incremental renal cell damage and eventual renal dysfunction.

The likelihood of developing an adverse health outcome related to pesticide exposure depends on a variety of factors, including route of exposure. Dermal exposure is thought to be the most common route of pesticide exposure in occupational settings (157), and exposure monitoring studies have found that the potential dermal exposure levels are many times greater than potential inhalation exposure levels across a variety of agricultural activities, pesticide formulations, and personal protective equipment types (158). Much is known about the adverse health effects of occupational dermal exposures (159), but research on the health effects of non-occupational dermal routes of pesticide exposure is limited. Prior research has not shown an association between laundering practices for pesticide-contaminated clothes and pesticide biomarkers (149). Risks were only modestly elevated for washing pesticide-contaminated clothing within twelve months of enrollment, though estimates were higher for women who reported no prior pesticide use. Though we did not see a clear trend with increasing frequency of washing such clothing, low numbers of cases and broad exposure categories limited our ability to observe an association if one exists.

Exposure monitoring studies have found detectable levels of pesticide degradates in groundwater and drinking water sources in the U.S. (36, 160). Investigators of chronic kidney disease of unknown origin (CKDu) suspect involvement of drinking water contaminated with heavy metals and/or pesticides in the etiology of the disease (21, 140), but epidemiologic research is limited in this area. We found some evidence of an association with having a private well near a pesticide mixing area, compared to no private

well or no mixing on site. It is unclear from these data whether this relationship is indicative of groundwater contamination or simply a marker for increased pesticide use activities by the applicator and his/her family.

Proximity to pesticide-treated farmland, as a surrogate for possible pesticide drift, is associated with higher detection rates and concentrations of common agricultural pesticides in household dust (25), but has not been consistently linked to higher levels of pesticides in urine or sera samples of women who live on farms (149)**.** We did not observe an association with closer proximity of the home to pesticide application areas in this study. However, at least one study has found detectable levels of pesticide concentrations in house dust in homes up to a quarter mile (440 yds) away from pesticide application areas (23). Thus, the lack of association in our study may be due to insufficient contrast between the exposed group (<100 yds) and the referent group (>300 yds). Additionally, whether pesticide particles drift from the treatment site to the home is highly dependent upon application method, pesticide formulation, and meteorology, which we were not able to evaluate in this study.

Wives who reported spending an average of 10 or more hours in the sun (compared to <1 hour) per day during the growing season 10 years before enrollment were at increased risk of ESRD diagnosis, and a significant exposure-response trend was observed for this risk factor. The weaker association with this factor for the growing season immediately prior to enrollment may reflect a latency period for development of disease. Because the question about time spent in the sun was not asked in the context of agricultural work, it is unclear whether it represents an occupational or leisure-time activity exposure, or both. Yet, those who spent more hours in the sun were more likely to engage in farming activities (i.e. apply chemical fertilizer, drive combines or other crop harvesters, hand pick crops, etc.) (data not shown). Women who were "in the immediate vicinity of pesticide activities" (161) in the Farm Family Exposure Study had modestly higher concentrations of pesticide biomarkers compared to women who were not present during pesticide application (162, 163). Thus, extended periods of time spent in the sun each day

during the growing season may be an indicator for increased potential for pesticide exposure through farm work activities or through spray drift while outside.

There were several limitations to this study. Despite the large sample size of the AHS cohort, evaluation of ESRD risk in relation to the wives' direct exposure was limited to 10 specific chemicals due to insufficient numbers of cases, and we could not adjust for husbands' use when evaluating associations with wives' use of specific chemicals. As a result, we may have failed to identify important associations between ESRD risk and less commonly used pesticides. Additionally, low case numbers resulted in construction of exposure categories that may not have provided enough contrast for adequate evaluation of the exposure-outcome relationship. For example, wives may handle pesticide-exposed clothing regardless of whether the clothes are washed in the same load as the family wash, washed separately in the same machine, or washed in a different machine. If wives are handling contaminated clothing in all scenarios, then the estimate of association for washing pesticide-exposed clothing with the family wash will be biased toward the null.

In order to evaluate indirect exposure to specific chemicals separately from direct exposure, we restricted analyses of husbands' use to women who reported that they never mixed or applied pesticides. Though this restriction resulted in limited power to evaluate exposure-response trends, we were still able to assess husbands' ever use of most reported chemicals in relation to ESRD risk. In addition, because only the decade of the husbands' first use was known for each pesticide, wives' estimated years of exposure to those chemicals may have been misclassified. Moreover, date of first use was imputed for 6% of applicators; however, complete case analyses (i.e. only using known data) produced similar results to analyses using imputed data, suggesting that our imputation did not bias results. Inaccurate recall of pesticide exposures by the wives or by the husbands may have also resulted in exposure misclassification. However, because outcome data were ascertained prospectively, exposure misclassification resulting from inaccurate recall is likely to be non-differential with respect to the outcome, thus biasing estimates toward the null. Though recall of pesticide use by the husbands' has been found to be reasonably reliable (136)

and accurate (145), validity of the wives' responses regarding pesticide use, farming activities, and residential pesticide exposures has not been assessed.

This study had several strengths. The large size of the cohort allowed for examination of an extensive range of potential occupational and non-occupational pesticide exposure pathways, none of which have been evaluated with respect to ESRD incidence among women. Our ability to take into account the number of years that the couple lived together in estimating wives' cumulative exposure to their husbands' pesticide use was an improvement upon prior methods used to assess health outcomes associated with husbands' cumulative use. We were also able to evaluate use of the five most commonly used pesticides by AHS women (148), all of which are still on the market and are readily available at home and garden stores across the country. Lastly, we had essentially complete case ascertainment and reliable data on ESRD diagnosis date.

Conclusions

While ever use of agricultural pesticides overall was inversely associated with ESRD risk, risk was elevated for personal use of several specific pesticides, and wives with the greatest cumulative lifetime-use of any pesticide had four times the risk of wives who seldom used pesticides. Additionally, potential indirect exposure to specific pesticides through the husbands' use may be associated with increased risk among women who do not apply pesticides, particularly for paraquat and butylate. Considering the widespread use of paraquat in developing countries (164), our findings in this and in our previous study of applicators have implications for agricultural workers and their families around the world. Other non-application factors such as time spent outdoors or laundering practices may also be relevant for the pesticide exposure-ESRD risk relationship; however, further research is needed to better elucidate the contribution of these and other pesticide exposures to overall ESRD risk in comparison to more traditional risk factors. Because this research is preliminary, and because many of our results are imprecise, additional studies are needed to confirm our findings.

Figure 4.1: Study population and numbers used for sub-analyses

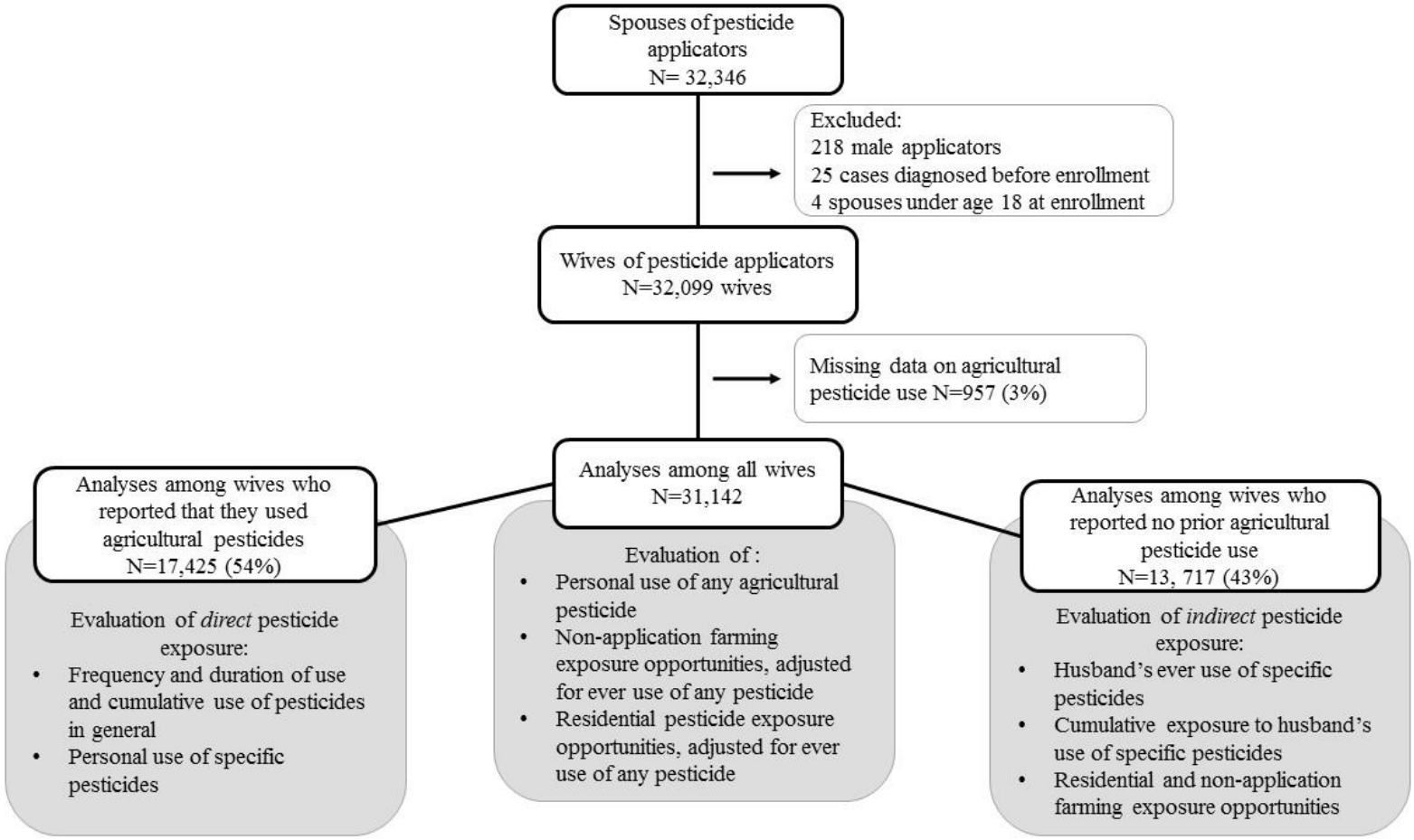


Table 4.1: Demographic and medical characteristics of all wives in the Agricultural Health Study, 1993-1997 through end of follow up (December, 31, 2011)

Characteristics (reported at enrollment)	Level	Non-cases N=31,996 (99.7%)		ESRD Cases N=103 (0.3%)	
		N	%	N	%
Age	18-39	9899	30.9	9	8.74
	40-49	9112	28.5	22	21.4
	50-59	7611	23.8	26	25.2
	60-69	4309	13.7	32	31.1
	≥70	1065	3.3	14	13.6
State	Iowa	21628	67.6	56	54.4
	North Carolina	10368	32.4	47	45.6
Education	< High School	18355	59.3	47	48.5
	High School	10786	34.9	40	41.2
	> High school	1800	5.8	10	10.3
Number of days per month drink alcohol	Never	13898	45.3	66	68
	< once a week	13092	42.7	21	21.6
	≥ once w week	3674	12	10	10.3
Pack years of smoking	None	21825	72.8	64	67.4
	>0 to 9	4812	16.1	16	16.8
	> 9	3337	11.1	15	15.8
BMI (kg/m^2)	<25	13792	49.2	29	34.1
	25-29.99	8998	32.1	31	36.5
	≥30	5227	18.7	25	29.4
Number of years take NSAIDS nearly every day	Have not taken nearly every day	20031	67.1	43	47.3
	< 1 year	3786	12.7	10	11
	1 or more years	6051	20.3	38	41.8
Diabetes	No	29583	96.8	44	45.8
	Yes	987	3.2	52	54.2
High blood pressure	No	25993	84.9	33	33.7
	Yes	4608	15.1	65	66.3

BMI = Body Mass Index

Table 4.2: Use of pesticides in general and specific pesticides by wives who reported using agricultural pesticides (direct exposure), adjusted for age, Agricultural Health Study, 1993-1997 through December 31, 2011

		Non-cases (N=17,391)		ESRD Cases (N=34)				
Risk factor	**Level**	**N**	**%**	**N**	**%**	**HR**	**95% CI**	**P for trend**
Cumulative lifetime-days of use of pesticides overall	0.1-24.5	5330	41.2	6	27.3	.	.	.
	24.6-98	2988	23.1	5	22.7	1.16	0.35, 3.83	
	98.1-507.5	3982	30.8	6	27.3	0.96	0.3, 3.034	
	>507.5	638	4.9	5	22.7	4.22	1.26, 14.2	
								0.024
Number of days per year personally mix or apply pesticides	0.1-2.5	6455	49.6	9	40.9	.	.	.
	2.6-7	2971	22.8	3	13.6	0.7	0.2, 2.54	
	7.1-14.5	2249	17.3	4	18.2	1.2	0.37, 3.88	
	>14.5	1339	10.3	6	27.3	2.8	0.99, 7.96	
								0.034
Number of years personally mixed or applied pesticides	<2	4750	36.5	6	27.3	.	.	.
	2-10	5538	42.6	5	22.7	0.66	0.2, 2.16	
	11-30	1584	12.2	3	13.6	1.11	0.27, 4.47	
	>30	1142	8.8	8	36.4	2.15	0.67, 6.86	
								0.155
Ever use of specific herbicides and herbicide chemical classes†								
Triazine herbicides	No	14847	89.8	27	90	.	.	
	Yes	1685	10.2	3	10	1.04	0.33, 3.22	
Atrazine	No	15150	91.8	27	90	.	.	
	Yes	1355	8.2	3	10	1.27	0.41, 3.96	
Chlorocetanilide herbicides	No	14762	89.9	26	86.7	.	.	
	Yes	1655	10.1	4	13.3	1.46	0.53, 4.02	
Alachlor	No	15176	92.3	26	86.7	.	.	
	Yes	1259	7.7	4	13.3	1.85	0.67, 5.12	
Phenoxy herbicides	No	12224	73.4	21	70	.	.	
	Yes	4438	26.6	9	30	1.1	0.5, 2.39	
2,4-D	No	12205	73.5	21	70	.	.	
	Yes	4405	26.5	9	30	1.11	0.51, 2.41	
Other herbicides								
Chlorimuron-ethyl	No	15880	96.8	27	90	.	.	.
	Yes	518	3.2	3	10	4.03	1.3, 12.51	
Glyphosate	No	6678	39.4	15	48.4	.	.	
	Yes	10281	60.6	16	51.6	0.83	0.41, 1.68	
Petroleum Oil	No	15335	93.5	27	90	.	.	
	Yes	1060	6.5	3	10	1.69	0.55, 5.24	
Imazethapyr	No	15484	94.5	27	90	.	.	
	Yes	902	5.5	3	10	2.37	0.76, 7.36	

Table 4.2 continued

Risk factor	Level	Non-cases (N=17,391) N	Non-cases (N=17,391) %	ESRD Cases (N=34) N	ESRD Cases (N=34) %	HR	95% CI
Ever use of specific insecticides and insecticide chemical classes*							
Pyrethroid insecticides	No	14543	91.1	26	89.7	.	.
	Yes	1423	8.9	3	10.3	1.57	0.5, 4.91
Carbamate insecticides	Yes	7179	42.8	9	28.1	.	.
	Yes	9590	57.2	23	71.9	1.5	0.69, 3.24
Carbaryl	No	7516	44.4	9	28.1	.	.
	Yes	9404	55.6	23	71.9	1.62	0.75, 3.5
Organophosphate insecticides	No	9377	54.5	18	58.1	.	.
	Yes	7834	45.5	13	41.9	0.78	0.38, 1.59
Diazinon	No	13398	81.4	26	83.9	.	.
	Yes	3068	18.6	5	16.1	0.88	0.35, 2.25
Malathion	No	10945	64.9	19	61.3	.	.
	Yes	5908	35.1	12	38.7	0.98	0.48, 2.03

†Herbicide chemical classes are: triazine herbicides (atrazine, cyanazine, and metribuzin); chlorocetanilide herbicides (alachlor and metolachlor); and phenoxy herbicides (2,4-D, 2,4,5-T, and 2,4,5-TP).

* Insecticide chemical classes are: pyrethroid insecticides (permethrin or pyrethroid products); carbamate insecticides (aldicarb, carbaryl, and carbofuran); and organophosphate insecticides (chlorpyrifos, coumaphos, diazinon, dichlorvos, fonofos, malathion, methyl or ethyl parathion, phorate, terbufos, and trichlorfon).

Table 4.3: Use of specific pesticides and pesticide chemical classes by husbands, among wives who did not apply agricultural pesticides (indirect exposure), adjusted for age, Agricultural Health Study, 1993-1997 through December 31, 2011

	Non-cases N=13,653 (43% of cohort)		ESRD Cases N=64 (62% of cases)			
	Exposed N	**Exposed %**	**Exposed N**	**Exposed %**	**HR**	**95% CI**
Ever use of fumigants and fungicides						
Benomyl	1510	11.5	3	5.1	0.42	0.14, 1.26
Metalaxyl	3162	24	17	27.9	1.2	0.68, 2.1
Captan	1336	10.3	4	7	0.79	0.3, 2.09
Chlorothalonil	1297	9.2	8	11.8	1.31	0.64, 2.71
Methyl Bromide	2327	16.5	14	20.6	1.19	0.66, 2.14
Carbon tetrachloride/ carbon disulfide (80/20 mix)	718	5.6	3	5.1	0.78	0.26, 2.33
Dithiocarbamate fungicides	1437	11.2	5	8.6	0.71	0.29, 1.73
Maneb	1392	10.7	5	8.5	0.74	0.31, 1.79
Ever use of specific herbicides and herbicide chemical classes						
Triazine herbicides	11204	77.4	51	75	0.95	0.55, 1.65
Atrazine	10144	71.6	45	68.2	0.89	0.53, 1.49
Cyanazine	5317	40.4	24	45.3	1.25	0.73, 2.15
Metribuzin	5763	44.5	29	50.9	1.34	0.79, 2.25
Chlorocetanilide herbicides	9190	69	43	76.8	1.58	0.85, 2.95
Alachlor	7228	54.6	38	67.9	1.68	0.96, 2.94
Metolachlor	6128	46.5	27	49.1	1.23	0.72, 2.08
Thiocarbamate herbicides	5058	40.3	29	55.8	1.85	1.07, 3.21
Butylate	4048	31.4	27	48.2	1.93	1.14, 3.26
EPTC	2488	19.2	8	15.4	0.87	0.41, 1.86
Dinitroaniline herbicides	9042	69.4	37	68.5	1.00	0.56, 1.77
Pendimethalin	5816	44.7	26	45.6	1.13	0.67, 1.91
Trifluralin	6908	52.2	30	55.6	1.16	0.68, 1.98
Phenoxy herbicides	11047	76.4	51	76.1	0.95	0.54, 1.67
2,4-D	10661	75.4	49	74.2	0.91	0.53, 1.58
2,4,5-T	2815	21.9	12	21.4	0.67	0.35, 1.29
2,4,5-TP	1170	9.1	5	9.1	0.84	0.35, 2.05
Other herbicides						
Chlorimuron-ethyl	4818	37.3	20	35.7	1.11	0.64, 1.93
Dicamba	6403	49	29	52.7	1.29	0.76, 2.2
Glyphosate	10619	74.7	47	71.2	0.92	0.54, 1.56

Table 4.3 continued

	Non-cases N=13,653 (43% of cohort)		ESRD Cases N=64 (62% of cases)			
	Exposed N	**Exposed %**	**Exposed N**	**Exposed %**	**HR**	**95% CI**
Paraquat	3208	24.8	25	44.6	2.33	1.38, 3.95
Petroleum Oil	6023	46.9	21	38.9	0.75	0.43, 1.3
Imazethapyr	5474	42	22	40	1.11	0.64, 1.92
Ever use of specific insecticides and insecticide chemical classes						
Pyrethroid insecticides	2985	23.1	8	14	0.68	0.32, 1.45
Permethrin (crops)	1708	13.2	3	5.5	0.52	0.18, 1.57
Permethrin (animals)	1573	12.0	5	8.6	1.00	0.41, 2.45
Organochlorines	7369	52.9	43	68.3	1.17	0.67, 2.03
Aldrin	2429	18.9	13	22.4	0.75	0.40, 1.42
Chlordane	3362	25.9	15	25.4	0.67	0.37, 1.21
DDT	3537	27	26	44.1	1.19	0.68, 2.08
Heptachlor	1946	15.2	10	17.9	0.76	0.38, 1.52
Lindane	2338	18.1	6	10.5	0.51	0.22, 1.15
Toxaphene	1878	14.7	10	17.5	0.86	0.43, 1.71
Carbamates	9353	66.1	40	62.5	0.7	0.42, 1.16
Aldicarb	1639	12.7	9	15.8	1.25	0.61, 2.54
Carbaryl	7398	55.5	34	56.7	0.88	0.53, 1.47
Carbofuran	3500	26.8	17	31.5	1.07	0.6, 1.90
Organophosphates	12791	88.4	55	80.9	0.59	0.32, 1.08
Chlorpyrifos	5925	41.9	19	28.8	0.63	0.37, 1.08
Diazinon	4082	31.5	13	22.4	0.57	0.31, 1.05
Dichlorvos	1166	9	9	15.5	1.86	0.91, 3.79
Fonofos	2740	20.8	14	25.9	1.34	0.73, 2.47
Malathion	9369	70.1	40	66.7	0.79	0.46, 1.36
Parathion	2057	16.1	5	8.8	0.46	0.19, 1.11
Phorate	4185	32.4	20	34.5	1.02	0.59, 1.75
Terbufos	5098	38.6	20	36.4	0.98	0.56, 1.70

Table 3.4: Relation between selected measures of potential farming and residential exposure to pesticides and risk of ESRD among all wives, adjusted for wives' ever use of any pesticide, Agricultural Health Study, 1993–1997 through December 31, 2011l

Risk factor	Time Frame	Level	Non-cases (N= 31142)		ESRD Cases (N=98)		HR	95% CI	
			N	%	N	%			
Ever personally mixed or applied pesticides?†		No	13653	44	64	65.3	.	.	
		Yes	17391	56	34	34.7	0.42	0.28, 0.64	
Farming pesticide exposure opportunities other than pesticide application activities									
Number of years lived or worked on farm over lifetime		0-28	13528	44.1	31	32.6	.	.	
		29-49	11102	36.2	31	32.6	0.81	0.48, 1.35	
		≥ 50	6024	19.7	33	34.7	0.8	0.46, 1.40	
									0.400
Number of days worked in field during the last growing season		None	14965	48.8	59	61.5	.	.	
		1-30	11415	37.3	24	25	0.71	0.44, 1.15	
		>30	4261	13.9	13	13.5	1.04	0.56, 1.92	
									0.708
Number of hours per day generally spend in sun during growing season	At enrollment	<1	6136	27	27	39.7	.	.	
		1-2	7360	32.4	18	26.5	0.67	0.37, 1.22	
		3-5	6455	28.4	15	22.1	0.67	0.36, 1.28	
		>5	2797	12.3	8	11.8	0.89	0.40, 1.97	
									0.643
	10 years before enrollment	<1	3785	17.7	15	22.4	.	.	
		1-2	5519	25.9	11	16.4	0.52	0.24, 1.13	
		3-5	7367	34.5	20	29.9	0.68	0.35, 1.33	
		6-10	3763	17.6	14	20.9	0.89	0.43, 1.85	
		>10	908	4.3	7	10.4	1.93	0.80, 4.71	
									0.042
Ever had an off-farm job		Had a job off farm	27354	89.2	75	77.3	.	.	
		Never had a job off farm	3303	10.8	22	22.7	1.46	0.9, 2.39	

Table 4.4 continued

Risk factor	Time Frame	Level	Non-cases (N= 31142) N	Non-cases (N= 31142) %	ESRD Cases (N=98) N	ESRD Cases (N=98) %	HR	Risk factor	Time Frame
Use of non-agricultural pesticides and residential pesticide exposure opportunities									
Usually treats home for pests		No	22289	71.8	72	73.5	.	.	
		Yes	8755	28.2	26	26.5	1.08	0.68, 1.71	
Usually treats lawn for pests		No	27650	89.1	90	91.8	.	.	
		Yes	3394	10.9	8	8.2	0.9	0.43, 1.88	
Clothes worn during pesticide mixing or application are washed with family wash	At enrollment (last 12 months)	No*	27161	89.3	81	87.1	.	.	
		Yes*	3260	10.7	12	12.9	1.09	0.59, 2	
	10 years before enrollment	No	22299	83.4	65	76.5	.	.	
		Yes	4436	16.6	20	23.5	1.53	0.93, 2.53	
Number of times per year personally wash clothes that were worn during pesticide application or mixing	At enrollment (last 12 months)	< 5	8155	34.2	27	35.5	.	.	
		5–10	6872	28.8	20	26.3	1.03	0.58, 1.84	
		11–15	3605	15.1	11	14.5	1.16	0.58, 2.36	
		16–20	2118	8.9	8	10.5	1.43	0.65, 3.17	
		>20	3091	13	10	13.2	1.36	0.65, 2.82	
									0.283
	10 years before enrollment	< 5	10829	36.6	40	44.4	.	.	
		5–10	8220	27.8	28	31.1	1.03	0.63, 1.67	
		11–15	4322	14.6	5	5.6	0.40	0.16, 0.99	
		16–20	2467	8.3	7	7.8	0.95	0.43, 2.09	
		>20	3770	12.7	10	11.1	0.99	0.49, 1.98	
									0.729

Table 4.4 continued

Risk factor	Time Frame	Level	Non-cases (N= 31142)		ESRD Cases (N=98)		HR	Risk factor	Time Frame
			N	%	N	%			
Pesticides stored in home	At enrollment (last 12 months)	No	11970	72.4	31	70.5	.	.	
		Yes	4572	27.6	13	29.5	1.13	0.59, 2.16	
	10 years before enrollment	No	9722	68.4	27	65.9	.	.	
		Yes	4491	31.6	14	34.1	1.17	0.61, 2.23	
Family members working in fields usually take work boots off before entering the house	At enrollment (last 12 months)	Yes	19353	63.6	61	64.2	.	.	
		No	11073	36.4	34	35.8	1.07	0.71, 1.64	
	10 years before enrollment	Yes	14703	59.9	48	60.8	.	.	
		No	9834	40.1	31	39.2	1.06	0.67, 1.67	
Distance from well to nearest area where pesticides were applied as of enrollment‡		Don't have private well/ no pesticides mixed on farm	3522	21.4	6	13.6	.	.	
		>100 yds	6073	36.8	16	36.4	1.34	0.55, 3.23	
		51-100 yds	4180	25.4	14	31.8	1.62	0.66, 3.98	
		≤50 yds	2714	16.5	8	18.2	1.49	0.55, 4.08	
									0.723¨
How far home from nearest field/ orchard where pesticides were applied	At enrollment (last 12 months)	≥300 yds	5630	21.1	19	27.1	.	.	
		100-299 yds	6370	23.9	14	20	0.68	0.34, 1.36	
		<100 yds	14664	55	37	52.9	0.88	0.51, 1.54	
									0.372
	10 years before enrollment	≥300 yds	4469	19.1	17	25.8	.	.	
		100-299 yds	5889	25.2	15	22.7	0.7	0.35, 1.40	
		<100 yds	13035	55.7	34	51.5	0.79	0.44, 1042	
									0.331

†Estimate is not adjusted for ever use

*No= Always use disposable clothing, clothes washed separately in family machine, washed in separate machine or sent out for cleaning; Yes = Clothes washed with family wash or soaked separately and then washed with family wash.

‡Data only available for wives whose husbands returned the take-home questionnaire

¨p for trend among those NOT in the referent category

Table 4.5: Associations between the husbands' cumulative lifetime use of specific chemicals and ESRD risk, among wives who reported no prior pesticide use, Agricultural Health Study, 1993–1997 through December 31, 2011

Pesticide	Lifetime-days of exposure	Non-cases N=13,653		ESRD Cases N=64		HR	95% CI	p for trend
		N	%	N	%			
FUNGICIDE								
Chlorothalonil	0	13040	92.6	61	89.7	.	.	
	>0.1-64	697	5	4	5.9	1.23	0.46, 3.23	
	>64	343	2.4	3	4.4	2.13	0.72, 6.34	
								0.1705
HERBICIDES								
2,4-D	0	4862	34.5	20	30.8	.	.	
	>0.1-87.5	5942	42.1	29	44.6	1.08	0.61, 1.9	
	>87.5	3297	23.4	16	24.6	0.9	0.46, 1.74	
								0.6232
Atrazine	0	5254	37.1	25	38.5	.	.	
	>0.1-56	5116	36.2	25	38.5	0.99	0.57, 1.72	
	>56	3776	26.7	15	23.1	0.72	0.38, 1.37	
								0.2807
Metribuzin	0	5672	72.6	23	69.7	.	.	
	>0.1-24.5	1578	20.2	6	18.2	0.99	0.4, 2.4	
	>24.5	561	7.2	4	12.1	1.92	0.68, 5.41	
								0.2187
Dicamba	0	7485	57.5	28	50.9	.	.	
	>0.1-24.5	2977	22.9	13	23.6	1.24	0.64, 2.41	
	>24.5	2550	19.6	14	25.5	1.45	0.76, 2.76	
								0.2872
Metolachlor	0	7854	59.8	30	55.6	.	.	
	>0.1-56	3689	28.1	14	25.9	1.02	0.54, 1.93	
	>56	1597	12.2	10	18.5	1.67	0.81, 3.43	
								0.1558
Alachlor	0	7153	54.3	23	41.1	.	.	
	0.1-50.8	3442	26.1	16	28.6	1.3	0.68, 2.46	
	>50.8	2585	19.6	17	30.4	1.7	0.91, 3.2	
								0.1137
Paraquat	0	6769	86.6	26	81.3	.	.	
	0.1-8.8	552	7.1	3	9.4	1.47	0.47, 4.59	
	>8.8	495	6.3	3	9.4	1.66	0.53, 5.19	
								0.3857
Petroleum Oil	0	6552	84.3	25	75.8	.	.	
	>0.1-24.5	674	8.7	5	15.2	2.26	0.88, 5.81	
	>24.5	545	7	3	9.1	1.57	0.5, 4.94	
								0.4462

Table 4.5 continued

		Non-cases N=13,653		ESRD Cases N=64				
Pesticide	**Lifetime-days of exposure**	**N**	**%**	**N**	**%**	**HR**	**95% CI**	**p for trend**
Pendimethalin	0	5517	70.5	24	72.7	.	.	
	0.1-50.8	1770	22.6	4	12.1	0.62	0.22, 1.73	
	>50.8	536	6.9	5	15.2	2.46	0.95, 6.36	
								0.0463
Imazethapyr	0	8738	67.3	35	63.6	.	.	
	0.1-21.8	3191	24.6	17	30.9	1.62	0.9, 2.91	
	>21.8	1056	8.1	3	5.5	0.91	0.3, 2.78	
								0.8686
Glyphosate	0	4972	35.1	28	42.4	.	.	
	0.1-38.8	5640	39.8	22	33.3	0.68	0.39, 1.19	
	>38.8	3551	25.1	16	24.2	0.78	0.42, 1.44	
								0.746
Butylate	0	6281	80.7	19	59.4	.	.	
	0.1-24.5	943	12.1	7	21.9	2.39	1.01, 5.68	
	>24.5	555	7.1	6	18.8	3.76	1.5, 9.39	
								0.0043
Trifluralin	0	7216	54.8	29	53.7	.	.	
	0.1-50.8	3142	23.9	15	27.8	1.16	0.62, 2.17	
	>50.8	2809	21.3	10	18.5	0.76	0.37, 1.57	
								0.42
INSECTICIDES								
DDT	0	6596	84.4	24	77.4	.	.	
	0.1-8.8	574	7.3	4	12.9	1.13	0.38, 3.33	
	>8.8	643	8.2	3	9.7	0.79	0.24, 2.62	
								0.8133
Fonofos	0	10817	82.3	44	81.5	.	.	
	0.1-24.5	1374	10.5	6	11.1	1.1	0.48, 2.53	
	>24.5	953	7.3	4	7.4	1.04	0.39, 2.79	
								0.9326
Chlorpyrifos	0	9119	64.6	49	74.2	.	.	
	0.1-50.8	3435	24.3	9	13.6	0.52	0.26, 1.07	
	>50.8	1571	11.1	8	12.1	0.97	0.46, 2.05	
								0.9413
Malathion	0	3865	49.5	14	46.7	.	.	
	0.1-8.8	1637	21	9	30	1.43	0.62, 3.31	
	>8.8	2300	29.5	7	23.3	0.74	0.3, 1.83	
								0.4107

Table 4.5 continued

Pesticide	Lifetime-days of exposure	Non-cases N=13,653 N	%	ESRD Cases N=64 N	%	HR	95% CI	p for trend
Phorate	0	6001	77.1	19	63.3	.	.	
	0.1-20	909	11.7	7	23.3	2.26	0.95, 5.36	
	>20	869	11.2	4	13.3	1.39	0.48, 3.97	
								0.5364
Dichlorvos	0	12033	92.7	50	86.2	.	.	
	0.1-44.4	503	3.9	4	6.9	1.99	0.75, 5.29	
	>44.4	443	3.4	4	6.9	2.21	0.83, 5.87	
								0.1154
Carbofuran	0	10098	77.4	44	81.5	.	.	
	0.1-31.6	2008	15.4	3	5.6	0.31	0.1, 0.95	
	>31.6	933	7.2	7	13	1.43	0.65, 3.13	
								0.3476
Carbaryl	0	5028	64.4	23	74.2	.	.	
	0.1-24.5	1488	19.1	4	12.9	0.55	0.19, 1.54	
	>24.5	1292	16.5	4	12.9	0.53	0.19, 1.51	
								0.303

Table 4.6 Associations between selected measures of potential non-application farming and residential pesticide exposure and risk of ESRD among wives who reported no prior pesticide use, Agricultural Health Study, 1993–1997 through December 31, 2011.

			Non-cases N=13,653		ESRD Cases N=64				
Risk factor	**Time frame**	**Level**	**N**	**%**	**N**	**%**	**HR**	**95% CI**	**p for trend**
Potential exposure to pesticides through farming									
Number of years lived or worked on farm over lifetime		0-28	6776	51.4	22	36.1	.	.	
		29-49	4113	31.2	19	31.1	0.83	0.44, 1.56	
		≥ 50	2285	17.3	20	32.8	0.91	0.46, 1.82	
									0.730
Number of days worked in field last season		None	8208	61.1	39	61.9	.	.	
		1-30	4119	30.6	15	23.8	0.87	0.48, 1.58	
		>30	1113	8.3	9	14.3	1.71	0.83, 3.54	
									0.123
Number of hours per day generally spend in sun during growing season	At enrollment	<1	3226	33.9	20	47.6	.	.	
		1-2	3121	32.8	10	23.8	0.6	0.28, 1.27	
		3-5	2304	24.2	6	14.3	0.49	0.2, 1.21	
		>5	866	9.1	6	14.3	1.32	0.53, 3.3	
									0.982
	10 years before enrollment	<1	2089	23.9	11	27.5	.	.	
		1-2	2398	27.4	6	15	0.51	0.19, 1.37	
		3-5	2740	31.4	10	25	0.74	0.31, 1.73	
		6-10	1191	13.6	8	20	1.27	0.51, 3.16	
		>10	321	3.7	5	12.5	3.1	1.08, 8.93	
									0.023
Ever had a job off farm		Had a job off farm	11853	88.2	48	76.2	.	.	
		Never had a job off farm	1586	11.8	15	23.8	1.58	0.87, 2.86	

Table 4.6 continued

Risk factor	Time frame	Level	N	Non-cases N=13,653 %	Non-cases N=13,653 N	ESRD Cases N=64 %	ESRD Cases N=64 HR	95 % CI	P for trend
Potential residential exposure to pesticides									
Usually treats home for pests		No	12041	82.4	58	84.1	.		
		Yes	2564	17.6	11	15.9	0.9	0.47, 1.71	
Usually treats lawn for pests		No	14112	96.6	67	97.1	.	.	
		Yes	493	3.4	2	2.9	-	-	
Clothes worn during pesticide mixing or application are washed with family wash	At enrollment (last 12 months)	No†	12192	89.8	52	83.9	.	.	
		Yes†	1391	10.2	10	16.1	1.42	0.71, 2.85	
	10 years before enrollment	No	9615	85	42	75	.	.	
		Yes	1698	15	14	25	1.88	1.03, 3.44	
Number of times per year personally wash clothes that were worn during pesticide application or mixing	At enrollment (last 12 months)	< 5	3908	41.2	20	42.6	.	.	
		5–10	2550	26.9	8	17	0.64	0.28, 1.46	
		11–15	1257	13.3	6	12.8	1.05	0.42, 2.63	
		16–20	718	7.6	6	12.8	1.77	0.71, 4.43	
		>20	1048	11.1	7	14.9	1.57	0.66, 3.72	
									0.142
	10 years before enrollment	< 5	5493	42.8	28	47.5	.	.	
		5–10	3331	25.9	15	25.4	0.96	0.51, 1.8	
		11–15	1622	12.6	3	5.1	0.43	0.13, 1.41	
		16–20	923	7.2	6	10.2	1.48	0.61, 3.59	
		>20	1468	11.4	7	11.9	1.22	0.53, 2.81	
									0.691

Table 4.6 continued

Risk factor	Time frame	Level	Non-cases N=13,653		ESRD Cases N=64		HR	95% CI	P for trend
			N	%	N	%			
Pesticides stored in home	At enrollment (last 12 months)	No	5735	75.7	25	83.3	.	.	
		Yes	1841	24.3	5	16.7	0.6	0.23, 1.57	
	10 years before enrollment	No	4484	72.2	21	77.8	.	.	
		Yes	1728	27.8	6	22.2	0.71	0.29, 1.77	
Family members working in fields usually take work boots off before entering the house	At enrollment (last 12 months)	Yes	8784	66.1	40	64.5	.	.	
		No	4514	33.9	22	35.5	0.83	0.49, 1.39	
	10 years before enrollment	Yes	6204	63	31	59.6	.	.	
		No	3644	37	21	40.4	0.78	0.45, 1.35	
Distance from well to nearest area where pesticides were applied as of enrollment*		Don't have private well/ no pesticides mixed on farm	1740	23	3	10	.	.	
		>100 yds	2849	37.6	9	30	1.91	0.52, 7.06	
		51-100 yds	1823	24.1	12	40	3.78	1.07, 13.4	
		≤50 yds	1167	15.4	6	20	2.93	0.73, 11.74	
									0.262
How far home from nearest field/ orchard where pesticides were applied	At enrollment (last 12 months)	≥300 yds	2995	25.8	10	23.8	.	.	
		100-299 yds	2680	23.1	7	16.7	0.83	0.32, 2.19	
		<100 yds	5923	51.1	25	59.5	1.5	0.72, 3.13	
									0.461
	10 years before enrollment	≥300 yds	2257	23.4	10	24.4	.	.	
		100-299 yds	2382	24.7	7	17.1	0.69	0.26, 1.82	
		<100 yds	5023	52	24	58.5	1.24	0.59, 2.59	
									0.733

† No= Always use disposable clothing, clothes washed separately in family machine, washed in separate machine or sent out for cleaning; Yes = Clothes washed with family wash or soaked separately and then washed with family wash.
* Data only available for wives whose husbands returned the take-home questionnaire

CHAPTER 5: Standardized Incidence Ratio Analysis

Introduction

In the previous chapters, we reported positive exposure-response relationships between ESRD risk and use of specific chemicals among pesticide applicators (Aim 1). Additionally, we observed elevated ESRD risk among wives whose pesticide applicator husbands use specific chemicals, compared to wives whose husbands do not use those chemicals (Aim 2). While these internal comparisons are useful for understanding which pesticides may increase renal disease risk among a population with relatively high pesticide exposure, they do not allow for comparison to a population that has a much lower pesticide exposure distribution. To assess whether risks were elevated among a population of pesticide users and their spouses compared to the general population, we calculated standardized incidence ratios (SIRs) comparing pesticide applicators and their spouses to the general populations of North Carolina and Iowa, overall and by state. Due to the healthy worker effect, in which occupational populations are healthier compared to the general population, comparative risk ratios between these two populations would likely be biased towards the null, and even down and through the null. In our study population, the HWE manifests as a lower prevalence of diabetes in the cohort compared to the general populations of Iowa and North Carolina; this difference in prevalence is even more pronounced within age strata. Diabetes is the most important risk factor for chronic kidney disease: approximately 40% of ESRD cases are attributable to diabetes. Therefore, another objective of this aim was to adjust for the low prevalence of diabetes in the cohort when estimating SIRs.

Methods

Data ascertainment and study populations

Using linked data from the United States Renal Data System (USRDS), we were able to identify virtually all incident ESRD cases occurring among AHS participants between enrollment (1993-1997) and December 31, 2011. The referent population (i.e. the population used for standardization) was made up of all persons living in NC and Iowa according to the 2000 and 2010 U.S. census. Census data for Iowa and North Carolina stratified by age, gender and race were not publicly available for years prior to 2000. We obtained annual incident ESRD counts for North Carolina and Iowa from 1994-2011 from publicly available data through the online USRDS Renal Data Extraction and Referencing (RENDER) system by age category (18-29, 30-39, 40-49, 50-59, 60-69, and ≥ 70 years of age), race (White vs. non-White), and gender. These data are accessible online at http://www.usrds.org/render/xrender_home.asp. Though ESRD data were also available through RENDER by calendar year, case numbers in the AHS were insufficient to further stratify by year. The applicator cohort is comprised of 52,394 private applicators and 4,916 commercial applicators. We did not want to include commercial pesticide applicators in SIR analyses of licensed applicators because commercial applicators differ from male private applicators with respect to age, lifestyle risk factors, use of personal protective equipment, types of pesticides used, and pesticide application methods (120). However, we could not analyze commercial applicators as a separate group because the small number of cases (N=17) would not permit standardization by age, an important risk factor for ESRD. Therefore, commercial applicators were excluded from these analyses.

The spouse cohort is comprised of 32,346 spouses of private pesticide applicators. Women have different ESRD risk factor distributions compared to men, and there were only three female applicator cases and four male spouse cases in the cohort. As such, we excluded female applicators and male spouses from study analyses, as well as cases diagnosed prior to enrollment and those under the age of 18 at enrollment. This left 50,920 male private applicators and 32,099 female spouses (i.e. wives) for

analyses. Analyses were conducted separately for applicators and wives because wives have a lower distribution of cumulative pesticide use compared to their applicator husbands.

Statistical analyses

Indirect standardization is preferred over direct standardization methods when the observed number of disease events in each standardization stratum in the study population is small. Because ESRD is a rare disease, we had small case numbers in age-by-sex and age-by-state strata; therefore we used indirect, rather than direct, standardization methods to compare the ESRD incidence experience of male licensed private applicators in Iowa and North Carolina to the total male population of Iowa and North Carolina. Though black race is associated with higher rates of ESRD, we could not include race as a standardizing factor in SIR analyses because there were no non-white ESRD cases in Iowa. Hence, overall estimates were standardized by age and state only, and the comparison population was restricted to white individuals. The referent population for analyses of applicators was comprised of 1.1 million and 3.2 million white males in Iowa and North Carolina, respectively, and the referent population for analyses of spouses was comprised of 1.1 million and 3.4 million white females in Iowa and North Carolina, respectively.

SIRs represent the comparison of two rates. Rates were calculated by taking the number of observed cases in a given stratum and dividing by the number of person-years accrued by all individuals in that stratum. Person-time was accrued through the first of exit from the age stratum, ESRD diagnosis, or death. Because census data for stratum-specific general population denominators were only available in 2000, and 2010, the average of the number of people in each age-by-state stratum represented the estimated populations from which cases arose between 1994 and 2011. This number was then multiplied by the average number of years of follow-up of the AHS cohort (15.6) to obtain person-years. We calculated summary SIRs from the total observed and expected counts and used Byar's approximation to the exact Poisson test to calculate 95% 2-sided confidence intervals (165).

In indirect standardization, weights used to obtain the standardized risks are the stratum sizes of the individual study populations. Therefore, if the age structure or the age stratum-specific ESRD rates

are very different in North Carolina vs. Iowa, then the summary SIRs for each state would not be comparable. In order to evaluate the comparability of state-specific summary SIRs, we reviewed the incidence rates in each age stratum and calculated age-specific SIRs for each state.

To avoid bias due to the healthy worker effect, researchers have suggested comparing the occupational cohort under study with a different occupational cohort, either within the same plant, or within the same industry (126). However, use of a comparison population with common characteristics or exposures, such as one from the same industry, could mask potential work-related exposure and underestimate the relative risk. An alternative option is to adjust the SIR for key risk factors if data on the prevalence of the risk factor and the risk ratio are available by age for the standard population and for the study population (133). Suta and Thompson (1983) provided an equation for calculating a smoking adjustment factor, which utilized data on the prevalence of smoking status (nonsmoker, former smoker, or current smoker) and the relative risk of lung cancer associated with smoking status:

Equation 5.1: Calculation of smoking adjustment factor, per Suta and Thompson, 1983

$$AD_{ijk} = \frac{\sum_{h=1}^{m} SA_{ijkh} \; x \; RR_{ijkh}}{\sum_{h=1}^{m} SG_{ijkh} \; x \; RR_{ijkh}}$$

where AD is the adjustment factor due to smoking differences; SA, fraction of automotive workers in the smoking category; SG, fraction of general population in the smoking category; RR, lung cancer mortality ratio due to smoking (i.e. dose-response relationship); h, indicator for smoking category; i, j, k, indicators for age, race, and sex, respectively; and m, number of smoking categories {Suta, 1983 #517}.

Diabetes is a strong risk factor for ESRD (22, 135), and prevalence of diabetes was lower in the cohort compared to the general population. Therefore, adjusting for diabetes could partially address the potential for the healthy worker effect to bias SIR estimates. Because there are only two levels of diabetes (i.e., diagnosed: yes/no), in using equation 5.1 for diabetes adjustment, we would not need to sum the numerator and denominator values over multiple values of the risk factor. Therefore, for diabetes adjustment, the equation would simplify to:

Equation 5.2: Calculation of diabetes adjustment factor

$$AD_{ij} = \frac{DBapp_{ij}}{DBgp_{ij}}$$

where AD is the adjustment factor due to diabetes differences; DBapp is the proportion of study participants reporting doctor-diagnosed diabetes; DBgp is the prevalence proportion of diabetes in the general population; and i and j represent age and state strata. The adjustment factor is then applied to the SIR calculations as follows:

Equation 5.3: Calculation of diabetes-adjusted standardized incidence ratio

$$Adjusted\ (SIR_{ij}) = \frac{\sum O\ (M_{ij})}{\sum E\ (M_{ij}) x\ AD_{ij}}$$

where O (M) is the observed number of ESRD cases among study participants, and E (M) is the expected number of ESRD cases based on the ESRD risk in the general populations of NC and IA.

Diabetes prevalence data were readily available by age group for the general populations of North Carolina and Iowa through the Behavioral Risk Factor Surveillance System, (BRFSS), which collects prevalence data via telephone questionnaire annually on more than 400,000 U.S. adult residents regarding their risk behaviors and preventive health practices. Post-stratification weights based on state population demographic characteristics were used to adjust for noncoverage and nonresponse. BRFSS participants were classified as having diabetes if they answered ‘yes’ to the question "Has a doctor ever told you that you have diabetes?" We used the 1995 BRFSS prevalence proportions of diabetes for adjustment analyses to compare to the diabetes prevalence of the AHS cohort at enrollment, which occurred between December 13, 1993 and November 06, 1997, with approximately even distribution of enrollment across years between 1994 and 1996.

We used PROC STDRATE in SAS 9.3 (Cary, NC) to calculate observed and expected ESRD cases by age stratum, by state, and overall SIR with 95% confidence intervals. PROC SURVEYFREQ

(SAS 9.3) was used to estimate weighted diabetes prevalence proportions by age, sex, and state. Other calculations were done manually in Microsoft Excel 2013.

Results

ESRD risk proportions by age and state are presented for the study populations and the general population in Table 5.1. The overall rate of ESRD in the study population was very similar to the overall rate in the general population for applicators (38.8 vs. 37.7 per 100,000 person-years) and wives (21.0 vs. 21.4 per 100,000 person-years). The summary SIR for applicators was 0.80 (95% CI: 0.72, 0.89), while age-specific SIRs ranged from 0.0 (NC aged 18-29) to 1.64 (IA aged 18-29). Among wives, the summary SIR was 0.70 (95% CI: 0.57, 0.84) with age-specific SIRs ranging from 0.0 (both states aged 18-29) to 1.62 (NC aged 40-49).

Because age-specific SIRs did not differ substantially by state (i.e. confidence intervals of one state included the SIR of the other state), we also calculated state-specific SIRs. The overall SIR for Iowa was lower than the SIR for NC for applicators (IA: SIR= 0.60, 95% CI: 0.50, 0.72 vs. NC: SIR = 1.04, 95% CI: 0.87, 1.21) and wives (IA: SIR=0.54, 95% CI: 0.41, 0.72 vs. NC: SIR= 1.04, 95% CI: 0.76, 1.39).

According to BRFSS data, diabetes prevalence in each state changed very little between 1994 and 1997, the last year of AHS cohort enrollment (data not shown). Diabetes prevalence in the AHS cohort was lower than in the general populations of IA (applicators: 2.0% and wives: 2.7% vs. 5.4% in IA), but similar to the general populations of NC (applicators: 3.9% and wives 4.4% vs. 4.5% in NC) in 1994. Adjustment for diabetes changed SIRs very little for both applicators (SIR = 0.77; 95% CI: 0.69, 0.87) and wives (SIR = 0.66; 95% CI: 0.54, 0.81) (Table 5.2).

Discussion

This is the first evaluation of the ESRD risk experience of a population occupationally exposed to pesticides compared to that of the general population. The age- and state-standardized risk of ESRD

among male private pesticide applicators and their wives in the Agricultural Health Study was significantly lower than the risk in the general populations of Iowa and North Carolina. Accounting for differential diabetes prevalence in the study and referent populations had little impact on SIR estimates. The SIR in NC was higher than that of IA, which may be related to the higher underlying ESRD rates in NC compared to IA.

Compared to SIRs observed in prior studies comparing morbidity and mortality rates in the AHS to the general population, the SIR we observed was similar to that observed for renal cancer but closer to the null compared to those observed for renal disease mortality. Waggoner et al (2011) found significantly lower mortality rates among AHS pesticide applicators compared to the general population for nearly all non-cancer internal causes of death, including renal failure due to acute glomerulonephritis (SMR=0.42; 95% CI: 0.17, 0.86), renal failure due to chronic and unspecified nephritis (SMR=0.54; 95% CI: 0.39, 0.73), and other genitourinary diseases (SMR=0.42; 95% CI: 0.25, 0.65) (132). Additionally, Koutros et al (2010) found significantly lower rates of kidney and renal pelvis cancers in the AHS private applicator population compared to the general population (SIR = 0.82; 95% CI: 0.69, 0.96) (169). The overall healthier lifestyle and disease profile of farmer pesticide applicators and their spouses compared to the general population may mask any excess risk due to occupational pesticide exposure if such an excess exists.

Our attempt to correct for this bias through adjustment for diabetes did not change the observed SIR. Blair et al (1985) also reported that adjustment for variability in smoking prevalence in an occupational cohort vs. the general population had little impact on the risk ratios for lung cancer (170). However, the lower rates of diabetes, hypertension, obesity, and smoking as well as higher levels of physical activity in the AHS cohort holistically contribute to lower ESRD incidence. Inability to adjust for all of these factors at once means that we may be missing a true excess in ESRD. In other words, if we could fully adjust for all of these factors, it is possible that the expected number of cases would be lower than the observed, resulting in an SIR>1.0.

Diabetes prevalence data were obtained from the BRFSS, which collected data via a land-line telephone questionnaire. These data were then weighted to represent the non-institutionalized populations of North Carolina and Iowa. We preferred to use BRFSS data over data from the National Health Interview Survey (NHIS) and the National Health and Nutrition Examination Survey (NHANES) because diabetes prevalence data were not readily available by state in the latter two surveys. If diabetes prevalence is underestimated in these populations and/or over-estimated in the AHS, the adjustment factor would be pushed towards the null and the correction to the SIR would be muted. Diabetes prevalence proportions in the BRFSS are comparable to those found in the NHIS and the NHANES (171). We used self-reported doctor-diagnosis of diabetes to determine diabetes status for both the study population and the general population. As a result of self-report, diabetes status may be misclassified; however we do not expect this misclassification to be differential by ESRD diagnosis, and Montgomery et al found a high level of reliability for self-reported doctor diagnosis of diabetes in the AHS cohort (1).

We used the age- and sex--stratified averages of the number of people living in North Carolina and Iowa from the 2000 and 2010 censuses as the referent population. Because we could not obtain exact estimates of population size in 1995, we may have over- or underestimated ESRD risk in the referent populations. However, due to the large size of these populations, any discrepancy between the estimated and actual population likely would have had little to no effect on our results. If some individuals living in the general population experience pesticide exposure levels similar to those of pesticide applicators, any difference we would have observed in ESRD incidence due to pesticide exposure would be reduced. The extent to which a lack of exposure contrast would affect our estimates remains unknown. Race is an important predictor of both ESRD (22) and diabetes (172), though at least one study found the fraction of ESRD risk attributable to diabetes to be similar in blacks (41%) and whites (44%) (58). We were able to adjust for race through restriction to whites, but doing so limited the generalizability of our results to non-white populations.

Despite these limitations, this is the only study to date to compare ESRD incidence among pesticide applicators and their spouses to the general population, and employed methods to address the

healthy worker effect. Attempts at comparing disease incidence in occupational cohorts to the general population are frequently limited by the lack of available data on disease rates by strata of behavioral risk factors or strongly associated medical conditions. Even if a reliable risk ratio can be identified, age-stratified risk ratios are often unavailable. In our study, we had age-stratified diabetes risk ratio and prevalence data for both the cohort and the general population, allowing for the calculation and use of a 'diabetes adjustment factor'. Without adjustment, one might attribute the observed SIR<1 to the differential prevalence of diabetes. Our ability to adjust for diabetes facilitated a more precise interpretation of study results. Though risk ratios were extrapolated for the youngest and oldest age groups, the diabetes adjustment factor was robust to changes in these estimates. Additionally, by using USRDS data, we had essentially complete case ascertainment in both the study and referent populations.

Summary

In summary, we observed a deficit in ESRD incidence among male private pesticide applicators and their wives in the AHS compared to the general population. The healthy worker effect related to diabetes prevalence did not appear to influence SIR estimates in this study, but other factors alone or in conjunction may play a role in biasing the SIR towards the null. Future research could attempt to adjust for other risk factors, and compare the ESRD risk experience of the sub-cohort of highly exposed pesticide applicators to that of the general population. As the cohort ages, there will be larger numbers of cases for analysis, which could also allow researchers to standardize by race. Additional investigation into the relationship between kidney disease and agricultural exposures will facilitate our understanding of the relative contribution of pesticide exposure to kidney disease etiology.

Table 5.1: ESRD risk proportions and crude standardized incidence ratios, comparing licensed private male pesticide applicators and their wives in the Agricultural Health Study to the general populations of Iowa and North Carolina.

Age	State†	Number of observed cases in the AHS	Study risk per 10,000	Reference population risk per 10,000	Expected number of events	Stratum-specific SIR*	95% CI
Pesticide Applicators							
18-29	IA	1	8.4	5.1	0.6	1.64	0.04, 9.13
18-29	NC	0	0	4.5	0.5	0.00	0.0, 0.0
30-39	IA	2	3.2	11.6	7.2	0.28	0.03, 1.00
30-39	NC	4	11.8	10.4	3.5	1.14	0.31, 2.91
40-49	IA	3	2.2	18.8	25.4	0.12	0.02, 0.34
40-49	NC	16	25	20.7	13.3	1.21	0.69, 1.96
50-59	IA	16	12.1	35	46.2	0.35	0.20, 0.56
50-59	NC	29	39	40.9	30.4	0.95	0.64, 1.37
60-69	IA	45	47.8	78.3	73.7	0.61	0.45, 0.82
60-69	NC	39	61.1	80.9	51.7	0.75	0.54, 1.03
≥70	IA	55	93.7	86.9	51.0	1.08	0.81, 1.43
≥70	NC	98	182.5	147.9	79.4	1.23	1.01, 1.51
Total		308			382.9	0.80	0.72, 0.9
Wives							
18-29	IA	0	0.0	3.1	0.1	0.00	0.0, 0.0
18-29	NC	0	0.0	2.0	0.0	0.00	0.0, 0.0
30-39	IA	2	4.9	7.5	3.1	0.65	0.08, 2.33
30-39	NC	1	6.3	4.8	0.8	1.32	0.03, 7.35
40-49	IA	4	4.3	11.3	10.6	0.38	0.10, 0.96
40-49	NC	5	14.5	8.9	3.1	1.62	0.52, 3.77
50-59	IA	15	16.3	24.7	22.7	0.66	0.37, 1.09
50-59	NC	5	11.8	20.4	8.6	0.58	0.19, 1.35
60-69	IA	18	27.1	56.7	37.6	0.48	0.28, 0.76
60-69	NC	15	40.4	43.8	16.3	0.92	0.52, 1.52
≥70	IA	17	49.1	80.4	27.8	0.61	0.36, 0.98
≥70	NC	21	78.2	60.9	16.3	1.29	0.80, 1.97
Total		103				0.70	0.57, 0.84

†IA = Iowa and NC = North Carolina
* SIR = Standardized Incidence Ratio

Table 5.2: Prevalence of diabetes and adjusted standardized incidence ratios, comparing incidence of end-stage renal disease in the Agricultural Health Study to that of Iowa and North Carolina

Age	State †	Prevalence (%) of DB* in AHS at enrollment	Prevalence (%) of DB in general population in 1995	Adjust-ment factor	Adjusted number of expected events	Adjusted SIR*	95% CI
Pesticide Applicators							
18-29	IA	0.00	0.01	0.45	0.28	3.62	0.09, 20.16
18-29	NC	0.01	0.01	0.74	0.34	0.00	0.0, 0.0
30-39	IA	0.01	0.01	1.43	10.28	0.19	0.02, 0.70
30-39	NC	0.01	0.00	2.82	9.93	0.40	0.11, 1.03
40-49	IA	0.01	0.01	1.48	37.63	0.08	0.02, 0.23
40-49	NC	0.03	0.03	1.21	16.01	1.00	0.57, 1.62
50-59	IA	0.03	0.03	1.04	47.83	0.33	0.19, 0.54
50-59	NC	0.07	0.06	1.15	35.07	0.83	0.55, 1.19
60-69	IA	0.05	0.05	1.05	77.25	0.58	0.42, 0.78
60-69	NC	0.10	0.08	1.14	58.72	0.66	0.47, 0.90
≥70	IA	0.07	0.11	0.64	32.59	1.69	1.27, 2.24
≥70	NC	0.10	0.11	0.91	72.25	1.36	1.11, 1.65
Total		2.94	IA = 5.4 NC =4.5‡		398.16	0.77	0.69, 0.87
Wives							
18-29	IA	0.01	0.01	0.59	0.08	0.00	0.0, 0.0
18-29	NC	0.01	0.01	0.81	0.04	0.00	0.0, 0.0
30-39	IA	0.01	0.03	0.33	1.02	1.97	0.24, 7.11
30-39	NC	0.01	0.01	2.34	1.77	0.56	0.01, 3.15
40-49	IA	0.02	0.00	3.64	38.68	0.10	0.03, 0.26
40-49	NC	0.03	0.02	1.47	4.54	1.10	0.36, 2.56
50-59	IA	0.04	0.06	0.66	14.88	1.01	0.56, 1.66
50-59	NC	0.05	0.07	0.76	6.54	0.76	0.25, 1.78
60-69	IA	0.08	0.12	0.65	24.52	0.73	0.44, 1.16
60-69	NC	0.09	0.07	1.25	20.26	0.74	0.41, 1.22
≥70	IA	0.10	0.09	1.03	28.78	0.59	0.34, 0.95
≥70	NC	0.12	0.14	0.88	14.31	1.47	0.91, 2.24
Total		3.24	IA = 5.4 NC =4.5†		155.42	0.66	0.54, 0.81

† IA = Iowa; NC = North Carolina
* SIR = Standardized Incidence Ratio; DB = Diabetes
‡An overall estimate of the total diabetes prevalence in Iowa and North Carolina in 1994 was not obtainable through the BRFSS online system

CHAPTER 6: Summary and directions for future research

The purpose of this dissertation was to advance our knowledge about the relationship between various pesticide exposure routes, types, and levels and the risk of end-stage renal disease. In addition to the limitations, strengths, and conclusions that were discussed with respect to each research objective in preceding chapters, the following discussion summarizes key study findings, addresses interpretation issues, and provides suggestions for future research.

Summary of findings and interpretation

Among a large cohort of male licensed pesticide applicators, several chemicals across various pesticide classes emerged as potentially important in relation to ESRD. Positive trends in ESRD risk with increasing cumulative use were observed for the herbicides pendimethalin and paraquat, the organophosphate coumaphos, the organochlorine chlordane, and the fungicide metalaxyl. The level of exposure was often lower for these chemicals than for other pesticides which were not associated with ESRD risk. For example, use of metalaxyl at an intensity-weighted exposure level $\geq$ 1680 lifetime-days was associated with a nearly two-fold increase in risk compared to no use, whereas use of 2,4-D for $\geq$ 6615 intensity-weighted lifetime-days was not associated with an increased risk. Increased risk with relatively low lifetime exposure levels may be an indicator for the relative toxicity of these chemicals. However, research on the direct toxicity to the kidney with exposure to these chemicals is rather limited and needs to be expanded in order to accurately interpret these findings.

One exception is paraquat. Due to the large number of reported paraquat poisonings (164), the literature describing the effect of exposure on the kidney is more robust for paraquat than it is for other chemicals. Acute kidney injury has occurred following paraquat intoxication (17, 173), and experimental evidence describes transport of paraquat through the renal tubules and subsequent damage to tubular and

glomerular renal cells (142, 155, 174, 175). Many paraquat intoxications result in death, but for those who survive, damage to the kidney may be irreversible. As such, given the potential for a feed-forward loop of kidney damage and dysfunction resulting from an initial kidney insult (55), and the increased risk of chronic kidney disease associated with acute kidney injury (98, 176), it is possible that a non-fatal intoxication episode or episodes may result in chronic renal dysfunction. There is some support for this hypothesis in our findings of significantly increased ESRD risk with hospitalization due to pesticide use and increasing risk within with increasing number of doctor visits due to pesticide use. Though unusually high pesticide exposure events (HPEE) and pesticide poisoning were not associated with ESRD risk, we had limited power to evaluate these exposures, particularly poisoning. Further, though HPEE may represent a subjectively high exposure, if the chemical that the individual was exposed to was of low toxicity, then such an event may not represent an exposure that is important for the pesticide-to-renal-damage pathway. By contrast, a visit to a medical professional may indicate a level of severity and/or toxicity that may have resulted in organ system damage, including kidney damage.

Spouses were also affected by paraquat. In our study, wives whose husbands ever used paraquat had twice the risk of ESRD compared to spouses whose husbands did not use this chemical. How exactly the husbands' use of this and other chemicals results in the types of exposures that can lead to health effects among wives remains unknown. However, there are several hypotheses for this pathway. For certain chemicals with low volatility and large droplet size (including paraquat), exposure is more common dermally than through inhalation (164). As husbands may track pesticide residues into the home on clothing, dermal exposure to pesticides through laundering pesticide-contaminated clothes may constitute an important exposure opportunity for wives. Additionally, proximity of the home or drinking water source to pesticide mixing and application areas may result in the presence of pesticide degradates in house dust or tap water. Prior research among wives of agricultural workers has not found higher levels of pesticide biomarkers to be associated with potential take-home or drift exposure (149), but these studies are unable to quantify lifetime exposures, which may be more relevant to the development of chronic disease. In our study we observed modestly elevated risks with closer proximity of one's private

well to pesticide mixing and with the practice of washing pesticide-exposed clothing with the family wash (vs. separately), particularly for wives who reported no prior personal pesticide use. Risk was also somewhat elevated for wives who reported washing pesticide-exposed clothes ≥ 16 vs. <5 days per year in the 12 months leading up to enrollment, but estimates were imprecise.

Though proximity of the home to pesticide application areas was not associated with increased risk, >10 hours spent in the sun each day during the growing season was associated with elevated risk, though again estimates were imprecise. Spending time in the sun during the growing season (whether for leisure or for occupational purposes) may be a proxy for being outdoors during pesticide exposure. In that way, wives may be exposed to pesticide drift over the lifetime, but more research is needed to better characterize frequency and duration of non-application exposures in relation to kidney disease. Smaller, more specific categories of exposure are available in the AHS dataset, but a larger number of cases will be needed to make use of these categories in future studies. Additionally, experimental studies are needed that evaluate the impact of different exposure routes (e.g. oral, dermal, inhalation, and ocular) and varying levels of paraquat and other chemicals on renal health. Such studies can help inform interpretation of the results in this and future epidemiological research on environmental and occupational risk factors for kidney disease.

Spouses were not asked about high pesticide exposure events or medical visits related to pesticide exposure, and there were no self-reported doctor-diagnosed pesticide poisonings among spouse cases. Therefore, we could not evaluate the extent to which spouses may be experiencing high levels of exposure, and the relationship to ESRD. Nonetheless, we did observe elevated ESRD risk among pesticide-applying wives for ever use of carbaryl, imazethapyr, alachlor, and chlorimuron-ethyl, though case numbers were very low for the latter three chemicals. Among spouses who reported no prior pesticide use, ESRD risk was significantly elevated with husbands' ever use of paraquat and butylate, and with >50.8 lifetime-days of use of pendimethalin vs. none. We observed positive-exposure response trends with the husbands' cumulative use of several chemicals, but these trends reached statistical significance only for butylate and pendimethalin. Because many chemicals that were associated with

ESRD among wives were not associated with ESRD among husbands and vice versa, results should be interpreted with caution. These discrepancies could be related to the pesticide formulation used, method of application, use of personal protective equipment, and size of the farm; however the impact of these factors on the relationship between pesticide exposure and ESRD risk is not yet known. Additionally, small cases numbers in spouse analyses did not permit the assessment of the wives' use of chemicals that were found to be associated with risk in applicator analyses. Further research into the mechanisms for renal damage and related dysfunction due to exposure to specific chemicals would help to contextualize these findings as potentially causal, or due to chance.

Results of our SIR analyses suggest that pesticide applicators experience a significant deficit in ESRD incidence compared with the general population. This is consistent with previous observations that farmers have a healthier lifestyle and lower prevalence of ESRD risk factors than the general population. Though adjustment for diabetes did not affect the SIR estimates, the healthy worker effect remains a potential source of bias. This healthy worker effect was reflected in our analyses of pesticide use among applicators and wives, in which we found that mixing or applying pesticides in general was not associated (applicators) or was inversely associated (wives) with ESRD risk, whereas exposure to specific chemicals and higher levels of cumulative use were associated with ESRD. Therefore, comparison of the entire cohort of pesticide applicators to the general population may be too crude to reflect the elevated ESRD risk experienced by sub-cohorts of pesticide applicators. Particularly because physical activity, smoking, and associated diabetes, hypertension and obesity are relatively strong predictors of chronic kidney disease, it will be difficult to adequately evaluate excess risk due to pesticide use in this cohort compared to the general population. Future studies with larger case numbers could evaluate relative risk (compared to the general population) for sub-cohorts of applicators who report heavy use of pesticides, use of more toxic chemicals, or longer term or higher frequency of pesticide use. Commercial applicators, a group with generally higher frequency of use, could also be analyzed separately.

Additional interpretation issues to consider

Although the mechanisms described are plausible means by which pesticides could contribute to the development of ESRD, further interpretation of the positive associations reported here requires consideration of measurement and design issues and other potential sources of confounding. Confounding by measured covariates was addressed through evaluation of potential confounders in relation to ESRD risk and the exposures of interest, and through inclusion of likely confounders in multivariable models; however, confounding by unmeasured variables is possible. For example, other agricultural exposures that are potentially predictive of ESRD may confound results if they are also predictive of pesticide exposure and on confounding pathways not blocked by other measured variables. Investigators have observed positive associations between ESRD risk and occupational exposure to solvents and silica (64, 66, 70, 177). However, results of these studies have been somewhat inconsistent, and comparisons across studies are difficult due to the varying study design and exposure definitions. In the AHS pesticide applicator cohort, we did not observe meaningful associations between ESRD risk and occupational exposure to silica and solvents. However, the AHS questionnaire variables may not adequately stand in as valid metrics of these exposures, and solvents have been and continue to be used in some pesticide formulations (178), which could partly explain the excess risk we observed for some chemicals. Additionally, among AHS applicators, ever use of petroleum oil was significantly associated with ESRD risk, but we did not observe a positive monotonic trend in risk with increasing petroleum oil use. It is possible that combined exposure to specific pesticides and solvents and/or silica increases ESRD risk beyond what has been observed for any of these agents alone. Future research on environmental risk factors for kidney disease could include evaluation of interactions between pesticide, solvent, and silica exposure in the development of kidney disease.

The present study made use of a new linkage between the AHS database and the United States Renal Data System (USRDS). Reporting of dialysis initiation using the Medical Evidence form 2728 was not required for non-Medicare-eligible (i.e. those covered by private insurance or Veterans Affairs)

patients prior to 1995; after 1995, form 2728 was required for all new ESRD patients regardless of Medicare status. Thus, it is possible that non-Medicare-eligible cases who enrolled in the Agricultural Health Study and were diagnosed prior to 1995 could be missing from the USRDS database and therefore classified as non-cases in this study. However, we expect few if any missing cases because most dialysis patients are eligible for Medicare at their first therapy visit, and diagnosis within 2 years of study enrollment is uncommon in this cohort; only 6% of cases in the study cohort were diagnosed within 2 years following enrollment.

A benefit of the study design was the planned internal comparison within the cohort for pesticide exposure analyses, thereby eliminating the potential for the healthy worker bias in analyses of applicators. However, conducting studies within occupationally exposed cohorts may mean that the group identified as unexposed has actually been exposed to other potentially nephrotoxic pesticides. This may have resulted in an underestimation of the association with certain chemicals if an association truly exists. The makeup of the spouse cohort provides the opportunity for more discrete comparisons of pesticide users to those with no prior pesticide use. But, evaluation of general pesticide use as a risk factor revealed an inverse relationship with ESRD risk, likely attributable to a more active lifestyle among pesticide-applying women. In order to avoid this healthy worker bias in analyses of ever/never use of specific chemicals, we excluded wives who did not apply pesticides, which limited our ability to make inferences to the general population. The spouse questionnaire did not collect data on cumulative use of specific pesticides at enrollment, but information on duration and frequency of use of specific pesticides (in the last year) was provided by spouses at Phase 2 (1999-2003). An opportunity for future research using Phase 2 spouse data could be to look at associations with frequency and duration of use of each pesticide compared to a truly unexposed referent group of non-applying spouses.

Another problem with internal comparison is the potential differential accrual of post-enrollment pesticide exposure by health status; unhealthy pesticide applicators and wives may select out of the occupation or activity that exposes them to pesticides, whereas healthy participants will continue to engage in work activities that expose them to pesticides. This healthy worker survivor effect (HWSE)

could mask the effects of pesticide exposure, biasing estimates towards the null. Though we could not evaluate differences in use or exposure following enrollment, we attempted to assess the impact that health status may have had on exposure prior to enrollment by excluding cases that were diagnosed within five years after enrollment. Participants diagnosed within 5 years of enrollment were probably already suffering from mild to moderately severe kidney disease or comorbidities of kidney disease at enrollment, which may have caused them to reduce the amount of pesticides they were using prior to enrollment. Results from this sub-analyses were very similar to those observed in the main analysis, suggesting a minimal impact of health status at enrollment on prior exposure.

In this study, we did not include post-enrollment exposure information, and therefore did not evaluate the association between recent exposures and ESRD. Progression of renal disease from chronic stage 1 to ESRD can take several decades; therefore, if pesticide use does contribute to kidney disease incidence, it is probable that this pathway would have been initiated prior to enrollment. Yet, depending on the intensity and frequency of exposure after enrollment, it is possible that exposure to nephrotoxic pesticides could accelerate kidney function decline among those with existing renal impairment. Future studies could address this question by incorporating pesticide exposure data from Phases 2 and 3 of the AHS and chronic kidney disease data from Medicare, though again such analyses may have limited power due to loss-to-follow-up in subsequent AHS phases.

Directions for future research

Although the results of the research presented in this dissertation are suggestive, a causal relationship between pesticide use/exposure and ESRD is far from proven. Further research is needed to clarify the mechanisms of injury and the development of clinical kidney disease related to pesticide exposure. Although our results suggest that acute exposure events leading to medical care is associated with incident kidney disease, additional information about these events would offer insights into mechanistic actions. Additional information could include: the exposure situation (e.g. spill/splash, leak, early re-entry, etc.), pesticide class and formulation, route of exposure and area of the body that was

exposed, whether care was sought, immediate health outcome of the exposure (e.g. mild dermal irritation, blistering, lesion, acute kidney injury, etc.), frequency of acute exposures, and time between acute exposures. To complement this research, experimental studies which focus on the specific outcomes of renal damage and dysfunction are needed for a broad range of pesticides, but particularly for alachlor, metalaxyl, pendimethalin, butylate, and coumaphos. Evaluation of the effects of prolonged low-level dosing on the renal systems of mammals could inform interpretation of results observed among spouses, who typically experience much lower levels of pesticide exposure compared to applicators. And, the relative contribution of dermal vs. inhalation exposure to kidney damage could facilitate understanding of non-occupational routes of exposure for family members of pesticide applicators.

Observed associations between pesticide exposure and ESRD have implications for the population at risk for developing earlier stages of kidney disease. Studies evaluating the association between pesticide exposure and earlier chronic kidney disease stages would benefit from increased statistical power relative to the research we conducted. Medicare claims data and self-reported CKD data provided by AHS participants at enrollment and subsequent AHS phases could be used to address this question. These data could also be used to evaluate the potential impact that pesticide exposure may have on progression of CKD.

There are many different subtypes of renal disease, some of which may be more likely to be associated with pesticide exposure. For example, prolonged exposure to nephrotoxic agents may eventually lead to permanent changes in the tubulointerstitium, such as tubular atrophy and interstitial fibrosis (4). Research among subjects with specific kidney diseases, where kidney biopsy is required for diagnosis, may provide an informative view into subtypes of disease or histopathological changes associated with pesticide exposure that would otherwise be unethical to obtain.

Limited evidence suggests that prenatal exposure to non-therapeutic chemicals can affect fetal kidney development (179-181), and may be a risk factor for the most common form of renal cancer in children (181). Abnormal fetal renal development related to maternal infection, malnutrition, and use of nephrotoxic drugs may become clinically relevant in adulthood (182). Thus, it is possible that disruptions

in renal development caused by *in utero* exposure to agrochemicals could increase the risk of kidney disease later in life. Follow-up of the children of AHS participants could provide valuable insight into the role of childhood or prenatal exposure to pesticides in the development of kidney disease.

Lastly, as evidence for environmental risk factors for kidney disease continues to surface, it will be important to look at combined environmental exposures in relation to kidney disease, particularly among farmers, who are potentially exposed to solvents, pesticides, and silica.

Public health significance

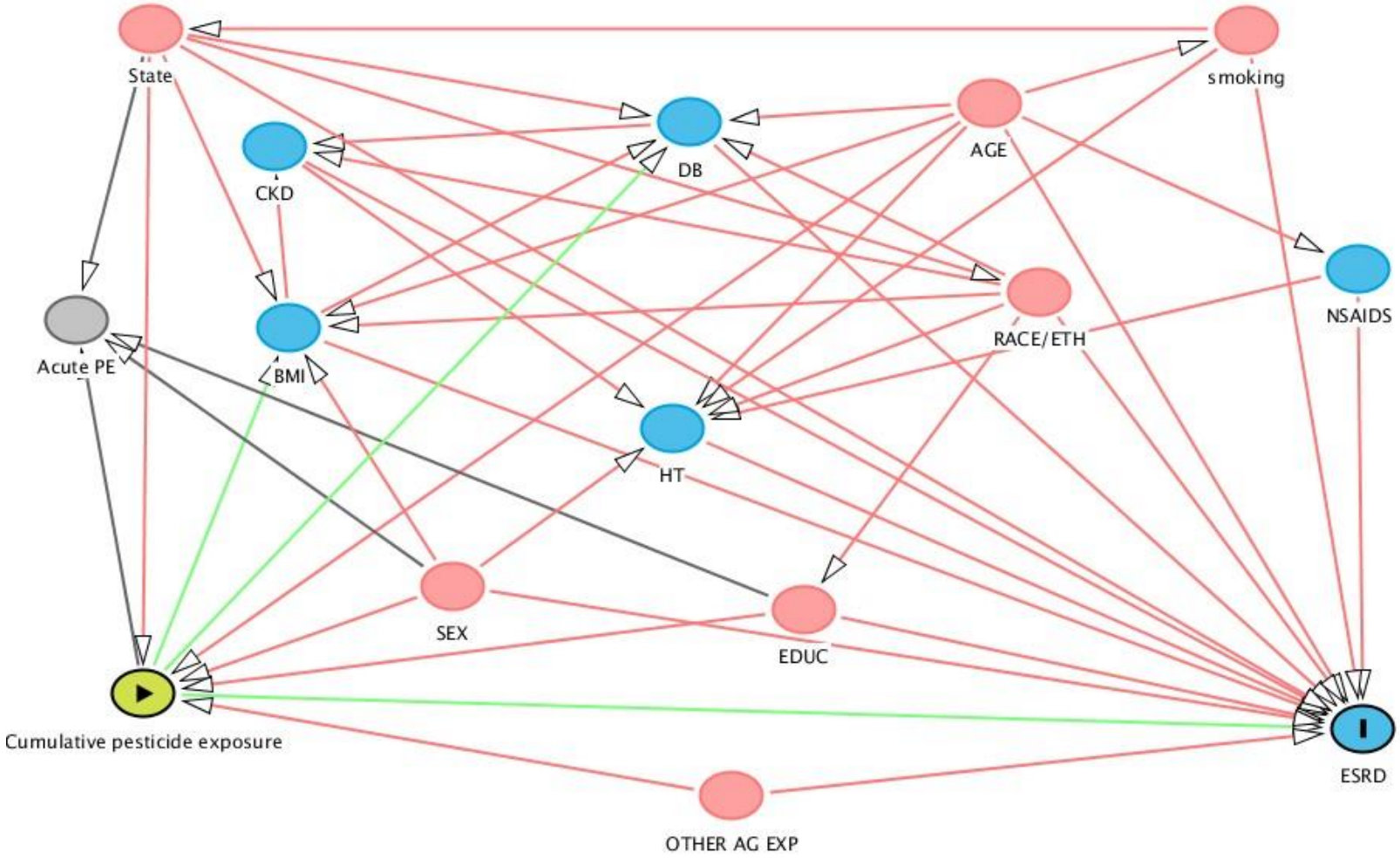
In evaluating environmental and occupational risk factors for renal disease, we have addressed a gap in the epidemiological literature which has not been able to characterize the role of long-term low level or acute non-fatal pesticide exposure in the development of renal disease. This relationship is of particular importance for populations living in developing countries, where most of the world's food is produced, where pesticide use regulations are often less stringent or non-existent, and where the incidence of chronic kidney disease continues to rise. Because access to renal replacement therapy is extremely limited in these countries (183), disease prevention strategies are paramount and can reasonably include removal of nephrotoxic agents from the environment.

The research presented in this dissertation also has implications for U.S. farmers and their families and for the millions of migrant farmworkers who often have limited access to health care. Identification of pesticide-related health risks and dissemination of information about these risks can inform individuals' decisions about use of specific pesticides, use of personal protective equipment, and household hygiene practices. Additionally, regulatory agencies can employ epidemiological study data to inform decisions about restricting or banning use of pesticides. Because non-farming populations living in close proximity to agricultural areas are also at risk of health effects due to pesticide exposure (184), identification and regulation of noxious chemicals may contribute to a reduction in pesticide-associated health effects for broader populations beyond pesticide applicators and their families.

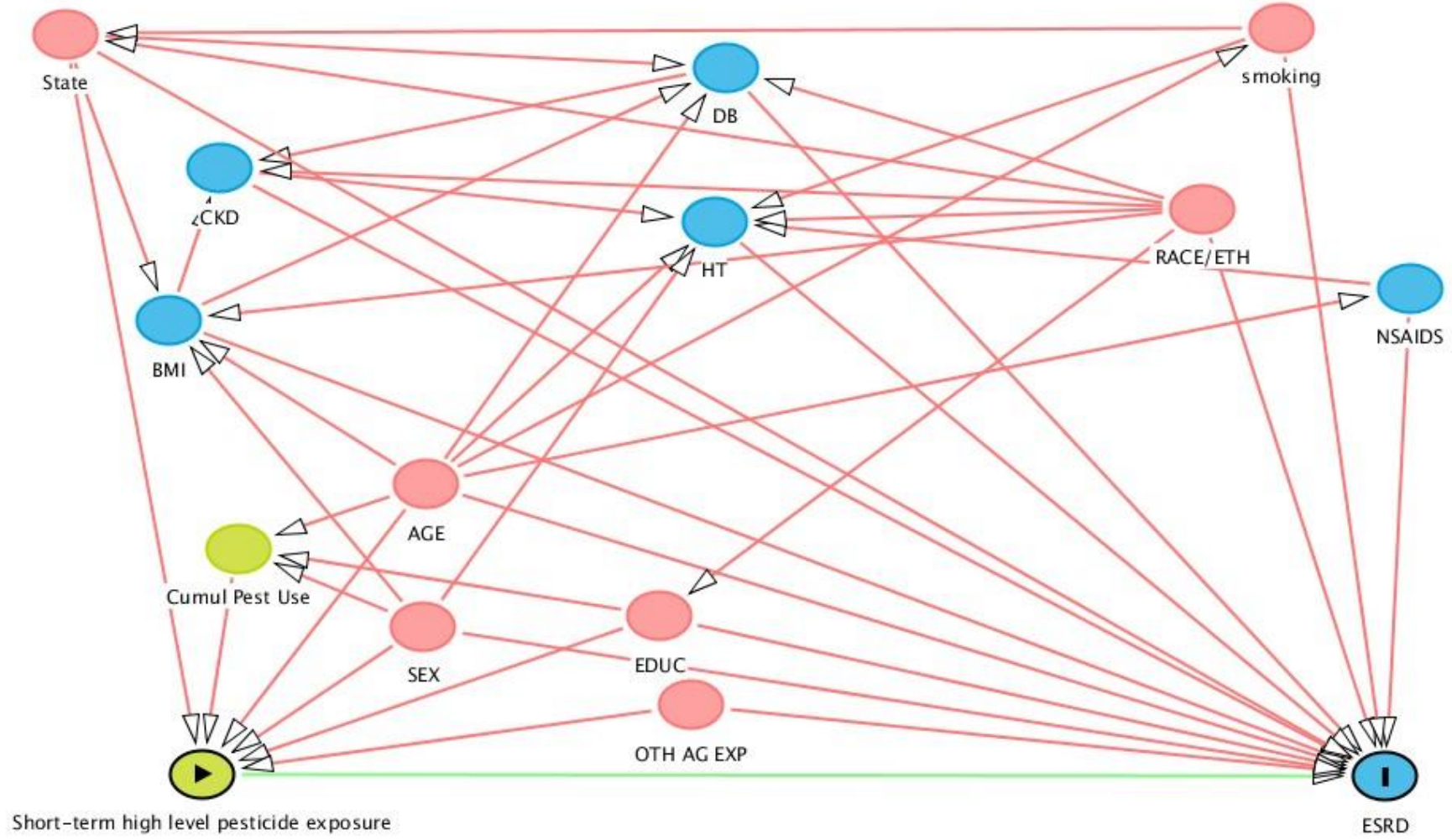
Though this research represents an important step in elucidating potential pathways for pesticide exposure in chronic renal disease, much additional research is needed to describe mechanisms for kidney damage and dysfunction with varying levels of specific pesticide exposure and to evaluate the relative importance of various exposure pathways for incurring exposure levels sufficient for disease causation. Additional epidemiological studies are also needed to confirm the findings of our research and to clarify the potential for ESRD risk related to pesticide exposure in non-agricultural populations. As a whole, this body of research could suggest new avenues for prevention of renal disease. Since the health effects and costs associated with ESRD are substantial, even a small reduction in ESRD incidence would have a large impact on the health and economy of the United States and other countries with high rates of disease.

APPENDICES

APPENDIX 1: Cumulative use of pesticides Directed Acyclic Graph



APPENDIX 2: Short-term high level pesticide exposures Directed Acyclic Graph



APPENDIX 3: Detailed literature review of animal studies of renal effects of pesticide exposure

Authors and year	Pesticide(s)	Class	Study type	Dose type	Histopathology	Biomarkers of kidney function	OS markers	DNA
Shah et al 2010	Diazinon	OP	Rat	10 mg/kg body weight, 15 mg/kg body weight and 30 mg/kg body weight daily for a period of 8 weeks	At all doses: kidney swelling with obliteration of space in Bowman's capsule, nuclear pycnosis, degeneration of tubular epithelial cells, necrosis of proximal tubules, flattened epithelium and congested blood vessels	sharp increase in blood urea nitrogen and serum creatinine.	Increased lipid peroxidation; Decrease in renal antioxidant enzymes (catalase, glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase, glutathione S-transferase) Increase in renal c-glutamyl transpeptidase and quinone reductase	N/A
Chargui et al 2012	Deltamethrin	Pyrethroid	Rat	subcutaneous injections of DM (at doses of 0.003, 0.03, and 0.3 mg/kg bw/d) after 30, 45, and 60 d, respectively	Lesions within proximal and distal tubules; alterations within glomeruli	No change in plasma creatinine or plasma urea	Increase of lipid peroxidation marker (MDA)	significant intense genotoxic alterations in lymphocyte DNA compared to controls.
Choudhary et al 2002	Endosulfan	Organochlorine	Rat	Or al dose: 10 mg /bodyweight/day for 15 and 30 days	Sclerosed glomerulus, necrosis of epithelial cells	Increased serum bilirubin, creatinine and urea	N/A	N/A

Authors and year	Pesticide(s)	Class	Study type	Dose type	Histopathology	Biomarkers of kidney function	OS markers	DNA damage
Kaur et al 2012	Carbofuran	Carbamate	Rat	(1 mg/kg body weight) orally for 28 days	N/A	Incrased serum creatinine and urea	decreased activity of antioxidant enzymes (superoxide dismutase and catalase) and increased lipid peroxidation	N/A
Tripathi et al 2010	Chlorpyrifos	OP	Rat	orally at a dose of 5 mg/kg b wt. and 10 mg/kg b wt for 8 weeks	shrinkage of glomerulus at initial stage of treatment, the tubular dilation, glomerular hypercellularity, hypertrophy of tubular epithelium, degeneration of glomerulus and renal tubules, deposition of eosin-positive substances in the glomerulus and renal tubules and infiltration of leucocytes			
Kackar et al 1999	Mancozeb	EBDC fungicide	Rats	500,1000,1500 mg/kg/day for 90, 180, and 360 days	Tubular necrosis, alteration of epithelial lining of convoluted tubules, degenerative changes in glomerular capsules		n/a looked at liver enzymes only	

Authors and year	Pesticide(s)	Class	Study type	Dose type	Histopathology	Biomarkers of kidney function	OS markers	DNA damage
Sonne et al 2008	Organochlorines*	OC	Foxes	395 ng/g wet weight in food for 2 years	glomerular, tubular and interstitial lesions	n/a	n/a	n/a
Eraslan et al 2009	Carbaryl	Carbamate	Rats	50, 100, and 225 mg/ kg b wt for 21 days	n/a	Increased creatinine	Signif increase in MDA (lipid peroxidation) in kidney tissues. Signif decrease in SOD and GSH-Px (glutathione peroxidase), increase in CAT	n/a
Oulmi et al 1995	Atrazine	Triazine herbicide	Rainbow trout	0, 10, 20, 40, 80, and160 ug/liter administered in water for 4 wks	Degeneration in tubular epithelial cells; ultrastructural alterations in renal tubule cells; cytological lesions	n/a	n/a	n/a
Alfaro-Lira et al 2012	Malathion	OP	Rats	22 mg/100 g BW; Injected for 5 days and then sacrificed at 30, 124, and 240	Increased Glomerular hypertrophy, and Signs of tubular damage,	n/a	n/a	n/a
Gill et al 1988	Dimethoate	OP	Fish	0.434 and 0.683 mg/L for dimethoate, being the i/llth and i/7th fractions of the 96-h LC50, respectively.	Degenerated epithelial cells in the renal tubules, distended renal tubules; collapsed glomeruli	n/a	n/a	n/a

Authors and year	Pesticide(s)	Class	Study type	Dose type	Histopathology	Biomarkers of kidney function	OS markers	DNA damage
Shao et al 2012	Endosulfan	OC	Zebrafish	(0.01, 0.1, 1, and 10 lg L-1) and were sampled after 7, 14, 21, and 28 days.	n/a	n/a	Low endosulfan concentrations (0.01 lg L-1) induced a slight increase of SOD and CAT activity, which kept ROS in a stable level. High endosulfan concentration (10 lg L-1) induced excessive ROS production which exceeded the capacity of the cellular antioxidants and exhausted the enzyme including CAT and SOD	DNA damage increased
Sobel et al 2005	chlordecone, methoxychlor, and o,p´-DDT)	OC	Rats	Implantation of subcutaneous sustained-release tablets (60 days). 0.01, 0.1, 0.5, 1.0 mg.	sclerotic glomeruli with tubular atrophy and dilation; chlordecone - significant proliferative glomerulonephritis with fibrosis,	Increased proteinuria; Exposure to all three pesticides significantly decreased the time to onset of renal impairment, as measured by urine protein and blood urea nitrogen	n/a	n/a

Authors and year	Pesticide(s)	Class	Study type	Dose type	Histopathology	Biomarkers of kidney function	OS markers	DNA damage
Ojha et al 2012	Chlorpyrifos, methyl parathion, and malathion	OP	Rats	One and two-day dose of: CPF: 15.5 mg and 38.8 mg; MPT: 1.3 mg and 3.3 mg/kg body wt; MLT: 137.5 mg andn/a 343.8 mg/kg body weight . These values correspond to 0.1 and 0.25 LD50s for these chemicals	n/a	n/a	Increased lipid peroxidation in kidney and other organs; significant decrease of catalase, superoxide dismutase and glutathione peroxidase in the kidney	n/a
Larsen et al 2012	Glyphosate "not an organophosphate ester but a phosphanoglycine, and it does not inhibit cholinesterase" from EXTOXNET	Phosphonate Herbicide	Rats	drinking water *ad libitum* with GLP at 0.7 mg/L during 30 and 90 days, and GLP at 7 mg/L for 30 and 90 days	absence of histomorphological changes in kidney tissues after GLP exposure through the drinking water	n/a	Significantly decreased glutathione s-transferase in kidney with highest dose, and significantly increased glutathione peroxidase in kidney at low and high doses. GLP exposure through the drinking water did **NOT** produce marked modifications in lipid peroxidation levels	n/a

Authors and year	Pesticide(s)	Class	Study type	Dose type	Histopathology	Biomarkers of kidney function	OS markers	DNA damage
El-Shanawy 2009	Glyphosate and Roundup	Phosphonate Herbicide	Rats	intraperitoneally treated with sub-lethal concentration of Roundup (269.9 mg/kg) or glyphosate (134.95 mg/kg) each 2 days,	n/a	No change in total protein levels or albumin. Significant decreases in creatinine were observed for glyphosate at one and two weeks and for roundup at two weeks	depletion of hepatic GSH, which indicates the activation of antioxidant defenses,	n/a
Uyanikgil et al2009	2,4,D	Chlorophenoxy herbicide	Rat	20, 40, 80 mg/kg by gastric gavage for 28 days	degeneration in renal corpuscles and podocytes; vacuolization in the glomerulus with disintegration of the basal membrane; tissue edema; vacuolization, cystic dilation and invagination of the basal laminae in the tubular structures; decrease in kidney weight ***dose-dependent histopathological degenerative effects in rat kidney cortex	n/a	n/a	n/a

*Sum of 1,3-DCB, 1,4-DCB, 1,2-DCB, 1,3,5-TCB, 1,2,4-TCB, 1,2, 3-TCB, Hexachlorobutadiene, 1,2,3,4-TTCB, PECB, a-HCH, HCB, Pentachloroanisole, b-HCH, g-HCH(Lindane), Heptchlor, Aldrin, Octachlorostyrene, Heptachlor epoxide, Oxychlordane, g-Chlordane, a-Endosulfan, o,p-DDE, a-Chlordane, trans-Nonachlor, Dieldrin, p,p-DDE, o,p-DDD, Endrin, b-Endosulfan, cis-Nonachlor, p,p-DDD, o,p-DDT, p,p-DDT, Methoxychlor, Mirex

APPENDIX 4: Frequencies and proportions of characteristics among prevalent and incident cases

	Incident Cases (N=423)		Prevalent Cases (N=67)	
Average number of years between ESRD diagnosis and death (SD)	2.7 (2.7)		11.9 (7.9)	
	N	**%**	**N**	**%**
Ever had a transplant				
No	340	80.38	15	22.39
Yes	83	19.62	52	77.61
Number of transplants				
0	340	80.38	15	22.39
1	81	19.15	36	53.73
2	2	0.47	14	20.9
3	0	0	2	2.99
Race				
Native Amer.	2	0.47	0	0
Asian	1	0.24	0	0
Black	58	13.71	6	8.96
White	362	85.58	61	91.04
Age				
<40	34	8.04	15	22.39
40 to <50	67	15.84	16	23.88
50 to <60	122	28.84	14	20.9
60 to <70	146	34.52	15	22.39
70+	54	12.77	7	10.45
Participant type				
Private applicator	308	72.81	37	55.22
Spouse	103	24.35	25	37.31
Commercial applicator	12	2.84	5	7.46
Education				
<High School	81	19.15	6	8.96
High School	108	25.53	33	49.25
>High school	203	47.99	24	35.82
Other	9	2.13	3	4.48
Body Mass Index				
normal	95	22.46	11	16.42
obese	139	32.86	22	32.84
overweight	1	0.24	0	0
Smoking status				
Never	185	47	37	58.7
Former	154	39	24	38
Current	55	14	2	3

APPENDIX 5: Exposure variable information

	Exposure being evaluated	Data source	Questionnaire item and categorization plan
Aim 2			
2A	Ever use of 50 pesticides	E	Have you ever personally mixed or applied this pesticide?
	Cumulative use (Lifetime days):		
	22 pesticides	E	Product of duration of use, i.e. ("How many years did you personally mix or apply this pesticide?") and frequency of use (i.e. "In an average year when you personally used this pesticide, how many days did you it?")
	28 pesticides	TH	
	Intensity-weighted lifetime days		
	22 pesticides	E	Product of the applicator's intensity level (114), duration of exposure to the pesticide, and frequency of application of the pesticide
	28 pesticides	TH	
2B	Pesticide poisoning	TH	Has a DOCTOR ever told you that you had (been diagnosed with) pesticide poisoning?
	High personal exposure to pesticides	TH	An incident or experience while using any type of pesticide which caused you unusually high personal exposure
	Hospitalization or visit to medical doctor due to pesticide use	E	As a result of using pesticides, how often have you seen a doctor/been hospitalized? Dichotomous: Never/Ever
Aim 3			
3A	Ever use of 50 specific pesticides	S	In your lifetime, have you mixed or applied the following pesticides
3B	Cumulative use of any pesticide	S	Product of "How many years did you personally mix or apply pesticides?" and "During those years, how many days per year did you personally mix or apply pesticides?"
3C	Indirect exposure		
	Cumulative use by applicator husband of :		Product of "How many years did you personally mix or apply this pesticide?" and "In an average year when you personally used this pesticide, how many days did you it?"
	22 pesticides	E	
	28 pesticides	TH	
	Number of days spent in the field during the growing season	S	During the last growing season, how many days per year did you work in the fields? None; <10 days; 10-30 days; 31-100 days; >100 days
	Ever had an off-farm job	S	Did you ever have a job off a farm? (Yes/No
	Farming activities during the last growing season	S	During the last growing season, did you do the following activities? (YES/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

	The following variables ask about exposures in the last 12 months, and ten years ago, separately		
	Exposure being evaluated	**Data source**	**Questionnaire item and categorization plan**
	Number of hours spent in the sun during the growing season	S	In the growing season, how many hours a day do you generally spend in the sun? Up to 1 hour; 1-2 hours; 3-5 hours; 6-10 hours; >10 hours
	Leave work boots on in house	S	Do family members who have been working in the fields usually take their work boots off before entering the house? (Yes/No)
	Mix contaminated clothes with family wash	S	In your household, how are clothes usually washed that have been worn when mixing or applying pesticides? Categories: always wear disposable clothing; mixed with family wash; not mixed with family wash
	Number of days per year wash contaminated clothes	S	*Among those who did not indicate 'always wear disposable clothing' on the previous question:* How many days per year do you personally wash clothes that have been worn during pesticide mixing or application? Categories: < 5 days; 5-20 days; more than 20 days
	Distance from home to nearest field or orchard where pesticides applied (yards)	S	How far is your home from the nearest field or orchard where pesticides are applied? Categories: < 100 yards; 101-300 yards; >300 yards Question also asked on TH for applicators, missing data can be supplemented from applicator TH questionnaire
E = enrollment questionnaire; TH= take-home questionnaire; S= spouse questionnaire			

APPENDIX 6: Review of the literature on relationships between ESRD risk factors and pesticide use/exposure.

ESRD incidence is higher in North Carolina vs. Iowa, among males, and among those with less than high school education. Farm size, distribution of pesticide use, and farming practices vary by state (113). In the AHS, farms are larger in Iowa, but pesticides are used more frequently by NC private applicators compared to IA private applicators. Pesticide application practices differ by state – for example, IA farmers are more likely than NC farmers to use a hand spray gun (61% vs. 48%), and NC farmers are more likely to use backpack sprayers than IA farmers (32% vs. 17%). Higher education level is associated with high pesticide exposure events (114, 120), but other pesticide exposures assessed in the AHS were not highly correlated with education (185). ESRD incidence increases with age, and age predicts pesticide use on the basis of the availability of certain pesticides over time, and the number of potential years of exposure to pesticides in general.

Heavy use of non-steroidal anti-inflammatory drug (NSAIDs) is associated with increased risk of CKD (52), ESRD (58), and with more rapid progression from CKD to ESRD (60). Though it is possible that increased pesticide use is associated with increased headaches or muscular pain, and thus correlated with increased NSAIDS use, this relationship is unknown and unlikely to bias the pesticide-ESRD relationship.

Hypertension can be controlled, and having uncontrolled hypertension should not affect the ability to mix or apply pesticides. Research on the association between pesticide use and hypertension is limited. Saldana et al (2009) observed higher risk of pregnancy-induced hypertension and preeclampsia among women who performed activities likely to have exposed them to pesticides during their first trimester of pregnancy, compared to those who did not (30). However, Goncharov et al (2011) did not find an association between use of chlorinated pesticides and hypertension (186). Without a clear relationship between pesticide exposure and hypertension, adjustment for hypertension was not necessary.

Diabetes is strongly associated with ESRD risk (135), and is the leading cause of ESRD, with approximately 40% of ESRD cases attributable to diabetes (27). Insecticide exposure, particularly for

certain organochlorines and organophosphates, has been associated with increased odds of diabetes (2, 3, 187, 188). In our study, frequency, duration, and cumulative lifetime-use of pesticides in general did not differ by self-reported diabetes status at enrollment. Lifetime pesticide use is elevated among applicators that are overweight or obese compared to those with normal BMI (189). There is some evidence to suggest that organochlorine and chlorpyrifos exposure increases the risk of overweight or obesity (5, 190), and obesity increases the risk of ESRD (22, 51). Given these findings, diabetes and BMI are likely to be on the causal pathway between pesticide exposure and ESRD. Adjusting for a factor that does not meet the criteria for confounding (i.e. is not associated with the exposure and/or is on the causal pathway between exposure and disease), also called 'over-adjusting', can reduce precision without reducing bias in measures of association; therefore, diabetes and BMI were not included as a confounders in analyses for Aims 1 and 2. However, standardized incidence ratio calculations (Aim 3) were adjusted for diabetes in order to account for the healthy worker effect.

Evidence of former or current smoking as a risk factor for chronic kidney disease is mixed (53). However, the literature does suggest that heavy smoking (i.e. 20 cigarettes per day or more) is associated with increased risk of CKD (51, 191-193). Smoking may increase the 'dose' of pesticide exposure if protective gloves are not worn and hands are not washed between pesticide mixing or applying and smoking activity. In this scenario, heavier smokers would be exposed to a higher pesticide dose compared to lighter smokers. However, smoking was not found to be associated with increased pesticide use in the AHS (185). Therefore, smoking is unlikely to confound the association between pesticide use and ESRD, and was not adjusted for in study analyses.

ESRD was found to be associated with occupational exposure to solvents and silica in non-AHS studies. Correlations between pesticide use, solvent use, and silica exposure were explored to evaluate these exposures as potential confounders. We did not observe correlation statistics >0.12 for correlations between duration and frequency of pesticide use and non-farm job exposure to solvents and silica, and these factors were not associated with ESRD. Thus, we did not adjust for solvent or silica exposure.

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