American Journal of Epidemiology

Vol. 161, No. 11
Printed in U.S.A.
DOI: 10.1093/aje/kwi140

Retinal Degeneration and Other Eye Disorders in Wives of Farmer Pesticide Applicators Enrolled in the Agricultural Health Study

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Received for publication August 13, 2004; accepted for publication February 1, 2005.

Retinal degeneration is the leading cause of visual impairment in older adults. An association between retinal degeneration and fungicide use was observed previously among farmer pesticide applicators in the Agricultural Health Study, a large study of farm families from Iowa and North Carolina. The objective of this investigation was to determine whether wives of these farmer pesticide applicators were at increased risk of retinal degeneration. Self-reported cross-sectional data obtained via questionnaire between 1993 and 1997 from 31,173 wives were used. Associations of specific pesticides and groups of pesticides based on function (fungicides, herbicides, insecticides, and fumigants) or chemical structure (organophosphates, organochlorines, and carbamates) with eye disorders were evaluated using logistic and hierarchical logistic regression analyses. Self-reported retinal degeneration was associated with the wife's fungicide use (odds ratio = 1.9, 95% confidence interval: 1.2, 3.1) after adjustment for age and state of residence. Specific fungicides that appeared to drive this association were maneb or mancozeb and ziram. No associations between pesticide use and other eye disorders were found. Although these findings for retinal degeneration are based solely on self-reported disease, they are consistent with those reported for farmer pesticide applicators. These findings suggest that exposure to some fungicides and other pesticides may increase the risk of retinal degeneration and warrant further investigation.

agriculture; eye diseases; occupational exposure; pesticides; retinal degeneration; spouses

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

The most common form of retinal degeneration, macular degeneration, is the leading cause of decreased visual acuity and loss of central vision among older adults in industrialized countries (1, 2). Known risk factors are age and family history. Smoking, hypertension, cardiovascular diseases related to atherosclerosis, cataract surgery, alcohol consumption, obesity, light exposure, and eye color are considered possible risk factors (1–3).

Exposure to pesticides may increase the risk of retinal degeneration. Studies in Japan have linked organophosphate exposure to Saku disease, which involves retinal degeneration and other adverse ocular effects (4, 5). In a study from India, the prevalence of macular degeneration was higher among pesticide workers exposed to the organophosphate fenthion than among unexposed controls (6). A link between organophosphates and retinal degeneration is supported by

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animal data (4, 7). Case reports have implicated organochlorines and carbamates in ocular toxicity (8, 9), and animal studies have implicated two fungicides, thiram and benomyl, in retinal degeneration (10, 11).

These findings suggest the need to study the role of pesticides in the etiology of retinal degeneration. An association of retinal degeneration with fungicides and other pesticides was observed among farmer pesticide applicators enrolled in the Agricultural Health Study (12), a large prospective study of more than 50,000 applicators and their family members in Iowa and North Carolina. Our objective in the current study was to investigate pesticides as a potential risk factor for eye disorders among applicators' wives, who may use pesticides themselves or be exposed to pesticides through indirect sources such as carry-home contamination or spray drift from nearby fields (13, 14).

MATERIALS AND METHODS

Population and questionnaires

The Agricultural Health Study has been described in detail elsewhere (15). Briefly, persons who applied for or renewed a license to buy Restricted Use Pesticides in Iowa and North Carolina between 1993 and 1997 were asked to participate. Approximately 52,000 private applicators, typically farmers, were enrolled (82 percent of those eligible). Of enrolled applicators, 81 percent were currently married, and we enrolled 76 percent of their eligible spouses.

A total of 31,173 wives whose husbands were the sole licensed pesticide applicator in the couple constituted our final study population. We excluded spouses of private applicators who did not own or work on a farm ($n = 996$) and male spouses of female applicators ($n = 178$).

We used cross-sectional data collected with two questionnaires, which are available on the Agricultural Health Study's website (www.aghealth.org). Most data came from the Spouse Questionnaire, which was self-administered and returned by mail. Some of the spouses who did not return their questionnaire by mail (19 percent of participating spouses) completed a modified version during a telephone interview. Additional data came from the Applicator Questionnaire, which was self-administered at the time of the applicator's enrollment.

Pesticide use was reported on the Spouse Questionnaire. Wives were asked whether they had ever mixed or applied any of 50 specific pesticides during their lifetimes. Agricultural use and residential use of pesticides were not distinguished. Groupings of specific pesticides were created based on similarities in function (fumigants, fungicides, herbicides, and insecticides) or chemical structure (carbamates, organophosphates, and organochlorines). No data were available that would allow the estimation of a dose-response relation for spouses. We did, however, have data from the Spouse Questionnaire on household hygiene factors thought to modify pesticide exposure, including family members' wearing work boots indoors or mixing pesticide-contaminated clothing with the family wash. The Applicator Questionnaire provided information on surrogate exposure measures (i.e., farm products and farm size) and husbands' use of pesticides.

The Spouse Questionnaire was also the source of information on personal characteristics and lifestyle factors potentially associated with retinal degeneration. Personal and lifestyle characteristics included age, state of residence, race, education, obesity, eye color, fruit and vegetable consumption, tendency to sunburn, employment off the farm, exercise, smoking, and alcohol consumption.

A doctor's diagnosis of retinal degeneration was reported on the spouse questionnaire. Persons who answered "yes" to the question "Has a doctor ever told you that you had retinal or macular degeneration?" were counted as cases. Cases of other eye disorders and medical conditions potentially associated with retinal degeneration were ascertained similarly. These other eye disorders and medical conditions were retinal detachment, glaucoma, cataract, diabetes, head injury, lead poisoning, solvent poisoning, pesticide poisoning, angina, arrhythmia, hypertension, myocardial infarction, and stroke.

Analysis

Logistic models were used to evaluate the associations of pesticide groupings with retinal degeneration. Two separate models defining pesticide use by function (four classes) and chemical structure (three classes) were specified. Each model included covariates and all of the classes. In secondary analyses, we explored the associations of pesticide groupings with detached retina, cataract, and glaucoma. The purpose of these analyses was to determine whether pesticides might pose a risk for these other eye disorders and to reveal a possible reporting bias that might affect all eye disorders.

We used two-stage hierarchical logistic regression to determine the associations of specific pesticides with retinal degeneration. These models included all 50 pesticides and were fitted using "semi-Bayes" methods (16). In two-stage hierarchical logistic models, the first stage is a logistic regression model. The second stage is a regression model that treats the parameters (beta coefficients) from the first stage as outcomes to be regressed on second-stage covariates. The second-stage covariates are chosen to incorporate knowledge about relationships among the first-stage parameters. This second-stage regression model implicitly defines a distribution for each first-stage parameter. An improvement in precision when using hierarchical logistic models as compared with simple logistic models arises, heuristically speaking, because honoring the second-stage model keeps the first-stage parameters from varying too widely. In effect, the usual logistic estimates are "shrunken" toward the relationships specified by the second-stage model. The magnitude of this shrinkage depends on the variance of the estimated parameters from the first-stage model and the prespecified residual variance for the second-stage model.

The first stage of our hierarchical model contained indicators of 50 specific pesticides and covariates. The second-stage covariates were indicator variables for the following hypothesized risk factors, identified through a literature review: 1) organophosphates, 2) organochlorines, 3) carbamates, and 4) fungicides. With this second-stage model, each first-stage estimate is shrunken toward the geometric mean of all the estimates in its own pesticide class.

TABLE 1. Relationship of retinal degeneration to characteristics of wives of farmer pesticide applicators, Agricultural Health Study, Iowa and North Carolina, 1993–1997*

Characteristic	% with characteristic		Adjusted OR†,‡	95% CI†
	Cases	Controls		
Personal characteristics				
Age (years)				
≤38	8.5	28.3	1.0§	
39–46	10.7	23.8	1.5	0.9, 2.4
47–56	22.1	25.3	2.9	1.8, 4.6
≥57	58.7	22.6	8.5	5.5, 3.0
State of residence				
Iowa	64.1	70.1	1.0§	
North Carolina	35.9	29.9	1.1	0.8, 1.3
Education				
Less than high school	7.6	5.3	0.7	0.4, 1.2
Completion of high school	46.2	40.4	1.0§	
More than high school	46.2	54.3	1.1	0.8, 1.4
Body mass index (kg/m^2)				
Normal (<25)	44.2	50.1	1.0§	
Overweight (≥25)	31.5	31.6	0.9	0.7, 1.3
Obese (≥30)	24.3	18.3	1.3	0.9, 1.8
Eye color				
Brown	27.7	27.9	1.0§	
Blue	41.7	37.8	1.1	0.8, 1.6
Hazel, gray, or green	30.6	34.3	1.0	0.7, 1.4
Skin reaction to first sun exposure each year				
No sunburn	15.2	16.7	1.0§	
Mild sunburn	47.5	48.4	1.4	1.0, 2.1
Severe sunburn	37.3	34.9	1.6	1.1, 2.4
Lifestyle characteristics				
Ever smoking cigarettes				
No	76.0	72.6	1.0§	
Yes	23.9	27.4	0.9	0.8, 1.3
Pack-years of smoking				
0	76.0	73.1	1.0§	
1–5	6.3	7.5	1.1	0.6, 1.8
6–15	5.5	8.8	0.9	0.6, 1.5
16–30	7.0	8.2	0.9	0.6, 1.5
≥31	5.2	2.4	1.2	0.7, 2.0
Alcohol consumption (drinks/month) in past year				
0	58.7	44.9	1.0§	
1–30	40.6	53.9	1.0	0.8, 1.3
>30	0.7	1.3	0.5	0.1, 1.9
Vegetable consumption (servings/week)				
0–4	9.3	18.3	1.0§	
5–10	41.5	43.8	1.6	1.0, 2.5
≥11	49.2	37.9	1.8	1.2, 2.9
Fruit consumption (servings/week)				
0–4	23.9	36.0	1.0§	
5–10	38.9	38.9	1.3	0.9, 1.8
≥11	37.2	25.1	1.6	1.1, 2.3
Summer exercise (hours/week)				
0	25.7	22.3	1.0§	
<1	17.5	17.7	1.0	0.7, 1.5
1–2	17.9	19.1	1.2	0.8, 1.7
3–5	21.8	25.0	1.1	0.7, 1.5
≥6	17.1	15.9	1.3	0.8, 1.9
Winter exercise (hours/week)				
0	28.7	26.9	1.0§	
<1	26.6	25.4	1.1	0.8, 1.6
1–2	18.5	22.2	1.1	0.7, 1.6
3–5	18.5	18.2	1.2	0.8, 1.8
≥6	7.7	7.3	1.3	0.8, 2.2
Ever worked off-the-farm				
No	16.0	10.8	1.0§	
Yes	84.0	89.2	1.1	0.8, 1.6

* Total number of cases = 233–281; total number of controls = 21,899–29,657 (variation due to missing values).

† OR, odds ratio; CI, confidence interval.

‡ Except for the model in which age was specified as a categorical variable and adjusted for state of residence, all potential confounders were modeled separately. Results were adjusted for age as a continuous variable and state of residence.

§ Referent.

TABLE 2. Relationship of self-reported retinal degeneration to other eye disorders and medical conditions among wives of farmer pesticide applicators, Agricultural Health Study, Iowa and North Carolina, 1993–1997*

Medical condition	% with characteristic		Adjusted OR†,‡	95% CI†
	Cases	Controls		
Detached retina	9.9	0.3	19.8	12.4, 31.7
Cataract	27.2	3.5	4.3	3.2, 5.9
Glaucoma	9.7	1.0	5.1	3.3, 7.9
Angina	9.7	2.3	2.4	1.6, 3.7
Arrhythmia	16.3	5.8	2.1	1.5, 2.9
High blood pressure	34.1	14.9	1.5	1.1, 1.9
Myocardial infarction	6.1	0.7	3.5	2.1, 5.9
Stroke	2.9	0.7	1.7	0.8, 3.6
Diabetes	17.2	3.2	3.5	2.5, 4.8
Head injury	7.0	4.6	1.6	1.0, 2.6
Solvent poisoning	0.4	0.1	3.1	0.4, 23.3

* Total number of cases = 270–279; total number of controls = 29,358–29,622 (range is due to missing values).

† OR, odds ratio; CI, confidence interval.

‡ Each medical condition was modeled separately. Results were adjusted for age (as a continuous variable) and state of residence.

We further assumed that the odds ratio for each pesticide would fall within a 10-fold range around its prior mean, with 95 percent certainty, by defining the prior residual variance as 0.35 (note: for a 10-fold range, residual variance = $((\ln(10)/3.92)^2 \approx 0.35))$ (16).

To evaluate potential confounding, we examined factors that have been previously associated with retinal degeneration or other eye disorders (table 1). If we detected an association between a potential confounder and an eye disorder, we included the potential confounder as a covariate in models that also contained variables for pesticide use, and we declared it a confounder if any pesticide effect changed by more than 10 percent. Age, as a continuous variable, and state of residence were included in all models. Age, diabetes status, cardiovascular disease status, state of residence, and husband's pesticide use were evaluated as potential effect modifiers and potential confounders. If differences across strata defined by these factors were observed, we constructed models including interaction terms for the relevant parameters. A significance level of 0.10 was used to determine whether interaction terms should be retained in the models. Additional covariates indicating whether family members wore work boots indoors and whether potentially contaminated work clothing was mixed with the family wash were considered in models containing husband's pesticide use.

We performed additional analyses to determine whether observed effects persisted in subpopulations. Our goal was to rule out alternative explanations for any observed pesticide effects. The subgroups included: 1) wives whose husband did not also report the same eye disorder; 2) wives

TABLE 3. Relationship of retinal degeneration to farm characteristics among wives of farmer pesticide applicators, Agricultural Health Study, Iowa and North Carolina, 1993–1997*

	% with characteristic		Adjusted OR†,‡	95% CI†
	Cases	Controls		
Current farm size				
<50 planted acres§	21.8	14.0	1.1	0.8, 1.6
Crop				
Grains	79.7	86.6	0.8	0.6, 2.9
Orchard fruit	6.1	2.6	1.6	0.9, 2.9
Other fruit	5.0	3.0	1.4	0.7, 2.8
Vegetables	9.6	8.3	0.7	0.4, 1.2
Christmas trees	2.5	2.1	1.1	0.5, 2.3
Cotton	2.5	3.8	0.8	0.3, 2.0
Peanuts	3.2	3.5	1.0	0.4, 2.1
Tobacco	12.8	12.8	1.1	0.7, 1.7
Animals				
Cattle	40.6	44.5	1.0	0.8, 1.3
Hogs	25.3	35.9	0.9	0.7, 1.2
Sheep/other animals	5.7	5.4	1.3	0.8, 2.2
Poultry	3.2	4.0	0.9	0.4, 1.7

* Number of cases = 257–281; number of controls = 29,657–27,836 (range is due to missing values).

† OR, odds ratio; CI, confidence interval.

‡ Variables for farm size, crops, and animals were entered into three separate models, each adjusted for age as a continuous variable and state of residence.

§ <20.2 hectares.

TABLE 4. Relationship of retinal degeneration to ever use of pesticides, classified by function or chemical structure, among wives of farmer pesticide applicators, Agricultural Health Study, Iowa and North Carolina, 1993–1997*

	% with characteristic		Adjusted OR†,‡	95% CI†
	Cases	Controls		
Functional class				
Fungicides	8.2	4.7	1.9	1.2, 3.1
Fumigants	1.9	1.7	0.4	0.1, 1.5
Herbicides	35.5	37.5	1.0	0.7, 1.3
Insecticides	41.5	39.3	1.0	0.7, 1.3
Chemical class				
Carbamate insecticides	35.0	31.3	1.0	0.8, 1.4
Organochlorine insecticides	12.9	7.6	1.2	0.8, 1.8
Organophosphate insecticides	29.1	25.5	1.1	0.8, 1.5

* Total number of cases = 267–271; total number of controls = 28,639–28,867 (range is due to missing values).

† OR, odds ratio; CI, confidence interval.

‡ Separate models were specified for the functional classes and the chemical classes, and each was adjusted for age and state of residence.

TABLE 5. Results from logistic and hierarchical regression analyses of the relationship of retinal degeneration to use of specific pesticides among wives of farmer pesticide applicators, Agricultural Health Study, North Carolina and Iowa, 1993–1997*

Pesticide active ingredient	% with characteristic		Hierarchical model	
	Cases	Controls	OR†,‡	95% CI†
Fumigants				
Methyl bromide	1.1	1.2	0.9	0.4, 2.2
Aluminum phosphide	0	0.12	0.9	0.3, 2.8
80/20 mix (carbon tetrachloride/carbon disulfide)	1.1	0.53	0.8	0.3, 2.2
Ethylene dibromide	0	0.12	0.9	0.3, 2.8
Fungicides				
Benomyl (also carbamate)	1.9	0.81	1.0	0.4, 2.7
Chlorothalonil	1.1	0.91	1.0	0.4, 2.7
Captan	3.4	2.1	1.0	0.5, 2.1
Maneb/mancozeb (also dithiocarbamate)	3.0	1.5	1.4	0.6, 3.0
Metalaxyl	1.5	1.5	1.1	0.5, 2.6
Ziram (also dithiocarbamate)	0.4	0.1	1.5	0.4, 5.0
Herbicides				
2,4,5-TP†	0.8	0.4	1.0	0.4, 2.9
2,4,5-T†	1	0.7	0.7	0.3, 1.9
2,4-D†	18.4	14.8	1.1	0.7, 1.8
Alachlor	5.2	4.3	1.2	0.6, 2.4
Atrazine	4.5	4.6	0.8	0.4, 1.7
Butylate (also carbamate)	1.5	1.4	0.9	0.4, 2.2
Chlorimuron ethyl	2.6	1.8	1.2	0.5, 2.7
Cyanazine	3.0	2.9	1.1	0.5, 2.4
Dicamba	4.8	4.1	1.1	0.5, 2.2
EPTC†	1.5	1.4	1.0	0.4, 2.5
Glyphosate	33	33.9	1.1	0.8, 1.5
Imazethapyr	4.8	3.1	1.7	0.8, 3.6
Metolachlor	2.6	3.4	0.7	0.3, 1.5
Metribuzin	2.2	1.8	0.9	0.4, 2.2
Paraquat	0.4	1.2	0.7	0.3, 1.7
Pendimethalin	1.9	2.5	0.8	0.4, 1.8
Petroleum oil	5.2	3.6	1.3	0.7, 2.5
Trifluralin	6.0	5.3	1.0	0.5, 1.9

Table continues

who reported only one eye disorder; 3) wives under the age of 57 years; and 4) wives whose husband did not report growing orchard fruit or other fruit, which was correlated with fungicide use.

We used the Agricultural Health Study data set released in July 2003 and SAS, version 8.0 (SAS Institute, Inc., Cary, North Carolina), for all analyses. We fitted logistic regression models with SAS Proc LOGISTIC and hierarchical regression models with SAS Proc GLIMMIX, adapted for hierarchical modeling of multiple exposures using a penalized likelihood function (16). Women with missing data on variables included in the models were excluded from the analyses. Therefore, the actual sample size (listed in the tables) for our analyses differed from the size of our total population ($N = 31,173$).

The institutional review boards of the National Cancer Institute and the National Institute of Environmental Health Sciences approved the Agricultural Health Study protocol. The study was explained to potential participants, and consent was signified by return of questionnaires.

RESULTS

Approximately 1 percent of the farm wives reported a doctor's diagnosis of retinal or macular degeneration

TABLE 5. Continued

Pesticide active ingredient	% with characteristic		Hierarchical model	
	Cases	Controls	OR‡	95% CI
Insecticides				
Carbamates				
Aldicarb	0.4	0.5	1.0	0.3, 3.2
Carbaryl	33.8	30.8	0.9	0.7, 1.3
Carbofuran	3.0	1.9	1.1	0.4, 2.8
Organophosphates				
Chlorpyrifos	3.3	4.0	0.8	0.4, 1.7
Coumaphos	0.8	1.3	0.5	0.2, 1.5
Diazinon	13.3	10.1	1.4	0.9, 2.2
Dichlorvos†	3.0	2.6	1.1	0.5, 2.2
Fonofos	1.5	1.9	0.9	0.4, 2.1
Malathion	23.8	19.5	1.0	0.6, 1.4
Parathion	0	1.0	0.6	0.2, 1.6
Phorate	1.9	2.0	0.9	0.4, 2.0
Terbufos	3.0	2.9	0.9	0.4, 2.1
Trichlorfon	0	0.2	0.8	0.2, 2.6
Organochlorines				
Aldrin	2.3	0.8	1.8	0.7, 4.4
Chlordane	7.5	4.2	1.3	0.7, 2.2
DDT†	7.5	3.6	1.0	0.6, 1.9
Dieldrin	<1	0.4	1.0	0.4, 2.9
Heptachlor	1.5	0.8	1.1	0.5, 2.8
Lindane	3.3	1.5	1.8	0.8, 3.9
Toxaphene	0.8	0.7	0.8	0.3, 2.2
Other insecticides				
Permethrin (crops)	2.2	2.0	0.9	0.4, 2.0
Permethrin (poultry)	1.9	3.5	0.8	0.4, 1.8

* $n = 26{,}809$ total observations.

† OR, odds ratio; CI, confidence interval; 2,4,5-TP, 2,4,5-trichlorophenoxypropionic acid; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; 2,4-D, 2,4-dichlorophenoxyacetic acid; EPTC, *S*-ethyl dipropylthiocarbamate; dichlorvos, 2,2-dichloroethenyl dimethylphosphate; DDT, dichlorodiphenyltrichloroethane.

‡ Fifty pesticides were modeled simultaneously. Results were adjusted for age and state of residence.

($n = 281$). Of the personal and lifestyle characteristics we examined (table 1), age was the strongest risk factor for retinal degeneration, and it was a strong confounder of the association between retinal degeneration and pesticides. Both experiencing severe sunburns and having a high fruit or vegetable intake (11 or more servings per week) were positively associated with retinal degeneration. Inclusion of these variables in models also containing indicator variables for classes of pesticides did not change the pesticide effects.

Other eye disorders and several medical conditions were positively associated with retinal degeneration after adjustment for age and state of residence (table 2). Detached retina was the most strongly associated. Conditions related to cardiovascular disease (i.e., angina, arrhythmia, hypertension, and myocardial infarction) and diabetes were also associated with retinal degeneration. There were no exposed cases of pesticide or lead poisoning, so these associations were not determined.

There were no strong associations between farm characteristics and retinal degeneration (table 3). Odds ratios for growing orchard fruit or other fruit were among the highest. When orchard fruit and other fruit were combined, the association with retinal degeneration was significant (odds ratio (OR) = 1.8, 95 percent confidence interval (CI): 1.1, 2.9). Fungicides were associated with a nearly twofold increased risk of retinal degeneration after adjustment for age and state of residence (table 4). No other functional or chemical class was associated with retinal degeneration. Farm sizes, types of crops grown, animals raised, and pesticide use differed according to state of residence, but the associations between these factors and retinal degeneration were similar in Iowa and North Carolina.

Subgroup analyses

Because a doctor's diagnosis of retinal degeneration may be confused with other eye disorders in self-reports, we conducted separate analyses excluding persons who reported retinal detachment, glaucoma, or cataract. After these exclusions, we lost precision but the elevated estimate for fungicides persisted (odds ratios were 1.6–1.8, depending on the eye disorder excluded). Since residual confounding by age may have influenced our results, we restricted analyses to women younger than age 57 years. The association of fungicides with retinal degeneration among the younger wives (OR = 1.7, 95 percent CI: 0.8, 3.6) was similar to that for all wives. A high prevalence of couples in which both husband and wife reported retinal degeneration may have revealed a reporting problem or a common exposure other than pesticides that could have skewed our results. However, when couples in which both husband and wife reported retinal or macular degeneration were excluded ($n = 8$; 0.03 percent), the association of retinal degeneration with fungicides among wives persisted (OR = 1.8, 95 percent CI: 1.1, 2.9).

We also conducted subgroup analyses to determine whether the associations of retinal degeneration with orchard and other fruit production and with fungicides were independent, since fungicides are commonly applied to fruit (particularly orchard fruit and berries). Among wives whose husbands did not report growing orchard fruit or other fruit, the odds ratio for fungicides was 2.3 (95 percent CI: 1.4, 3.8). The odds ratio for growing orchard or other fruit was 2.2 (95 percent CI: 1.3, 3.6) among wives who reported never using fungicides. However, the odds ratio for growing orchard or other fruit was not significantly elevated when the analysis was restricted to couples where both the wife and the husband never reported using fungicides (OR = 1.2, 95 percent CI: 0.5, 3.0).

Cardiovascular disease and diabetes might increase a pesticide-exposed person's susceptibility to retinal degeneration if these conditions weaken structures in the eye such as Bruch's membrane or the retinal pigment epithelium (3). The association of retinal degeneration with fungicides among persons reporting conditions indicative of cardiovascular disease (i.e., angina, arrhythmia, hypertension, myocardial infarction, or stroke) was stronger (OR = 2.2, 95 percent CI: 1.1, 4.3) than that among persons who did not report these diseases (OR = 1.7, 95 percent CI: 0.8, 3.3), but confidence intervals overlapped. We observed a large difference in the effect of fungicides depending on whether the wife also reported diabetes and a significant interaction between diabetes and fungicide use. The odds ratio for fungicides among wives with diabetes was 14.8 (95 percent CI: 3.1, 69.8), but this result was based on a small number of wives with both exposure and disease. The odds ratio for exposure to fungicides among wives without diabetes was 1.6 (95 percent CI: 0.9, 2.8).

Specific pesticides

We used hierarchical regression models to identify specific pesticides that were associated with retinal degeneration (table 5). All 50 pesticides were included in one hierarchical model with results adjusted for age and state of residence. The covariates included in the second-stage model were indicator variables for groupings of fungicides, carbamates, organophosphates, and organochlorines, which have been linked to retinal degeneration in some reports. Of the fungicides, the specific chemicals with increased odds ratios were maneb or mancozeb and ziram. The odds ratios for imazethapyr, lindane, and aldrin were elevated, but not significantly. There were too few exposed wives to conduct analyses of potential interactions between individual pesticides.

Husband's pesticide use

We saw no statistical interaction between the wives' and the husbands' pesticide use (functional class model). In addition, we observed no confounding of the association between wives' pesticide use and retinal degeneration by husbands' pesticide use (functional class model). Husbands' pesticide use was not associated with wives' retinal degeneration among unexposed women; nor did wives' retinal degeneration vary across strata defined by family members' wearing work boots indoors or mixing pesticide-contaminated clothing with the family wash.

Cataract, retinal detachment, and glaucoma

We also evaluated the relationships of retinal detachment ($n = 133$ cases), glaucoma ($n = 332$ cases), and cataract ($n = 1{,}115$ cases) to pesticide use. We found no significant associations of these eye disorders with lifetime use of pesticides for any of the functional or chemical classes we considered (data not shown). Nonsignificant associations of retinal detachment with fungicides (OR = 1.8, 95 percent CI: 0.8, 3.7) and organochlorines (OR = 1.4, 95 percent CI: 0.8, 2.7) decreased to 1.4 (95 percent CI: 0.6, 3.5) and 1.2 (95 percent CI: 0.3, 5.0), respectively, after cases of retinal degeneration were excluded.

DISCUSSION

Fungicide use is rare in the general population, but these chemicals are commonly used on farms (33 percent of farms in the Agricultural Health Study). We found a positive association between retinal degeneration and ever use of fungicides by wives of farmer pesticide applicators enrolled in the Agricultural Health Study. This association is similar in magnitude and direction to that reported by Kamel et al. (12) for farmer pesticide applicators in the Agricultural Health Study (OR = 2.0, 95 percent CI: 1.1, 3.6). Both studies relied on self-reported information on retinal degeneration without physician confirmation. While this is likely to have resulted in misclassification of disease status, it is unlikely to explain the consistent positive findings with fungicides in the cohort. Prior to the analysis of the farmer data, there was no evidence to suggest an association between fungicides and retinal degeneration, so recall bias is also unlikely to explain these results.

For pesticide applicators, the association between retinal degeneration and orchard fruit production diminished when analyses were restricted to persons who did not use fungicides (12). This was not the case for wives, for whom the association with growing orchard fruit or other fruit remained after the analysis was restricted to wives who did not use fungicides. This difference may be partly explained by the possibility that indirect exposure to fungicides occurs when pesticides are carried home by the applicator or through spray drift from the field. The association between growing orchard fruit or other fruit and wives' retinal degeneration did not persist if neither the husband nor the wife reported fungicide use. This finding reduces the likelihood that unmeasured factors associated with orchards or fruit-growing, other than fungicide use per se, are responsible for the association between retinal degeneration and orchard fruit or other fruit. Since applicators who raise orchard fruit tend to be older, there could also be residual confounding by age. However, when we specifically evaluated applicators under age 57, the effect of fungicide exposure remained.

Applicators' use of fungicides was not independently associated with wives' retinal degeneration in these data. Although we had limited ability to evaluate the contribution of husbands' exposure to household contamination, the association for wives' retinal degeneration and fungicide use did not differ by household hygiene factors that could influence residential contamination.

No risk factors other than age and family history have been associated consistently with retinal degeneration (1, 3, 17). Age was strongly related to retinal degeneration in Agricultural Health Study farm wives. Retinal degeneration was also associated with severe sunburn. This association has been reported previously (1, 3, 17). The wives were relatively homogenous with regard to eye color and smoking patterns and the prevalence of ever smoking was low, limiting our ability to detect associations. In our analyses, eye color and cigarette smoking were not associated with retinal degeneration among the wives.

We observed an association between retinal degeneration and high intake of fruits and vegetables. Older wives in this population consumed more fruits and vegetables than did younger wives. These women may have changed their diet in response to a diagnosis of retinal degeneration or other age-related disease.

Retinal degeneration was also associated with other eye disorders. These associations may suggest a common disease pathway or indicate that persons who see a doctor for one eye condition are more likely to be diagnosed with another condition. An association between cataract surgery and macular degeneration has been reported (1, 3). Similarly, retinal detachment may be a consequence of retinal degeneration or may result from other common causes (18). Fungicide use was not associated with cataract and glaucoma among wives enrolled in the Agricultural Health Study, which suggests that a common disease pathway involving fungicides is not likely. These negative findings for other eye disorders also suggest that our results are not entirely due to biased reporting of our outcomes.

Associations of retinal degeneration with several types of cardiovascular disease were also observed. High blood pressure has been inconsistently linked to retinal degeneration in epidemiologic studies. Although preclinical signs of atherosclerosis are associated with retinal degeneration (19), clinical manifestations of this disease, such as stroke or myocardial infarction, have not been associated with retinal degeneration in most studies (3).

We found a strong association between retinal degeneration and diabetes (OR = 3.5, 95 percent CI: 2.5, 4.8). All of eight published studies recently reviewed reported no positive association between macular degeneration and diabetes (3). Since all of our data were self-reported, women may have confused a diagnosis of diabetic retinopathy with retinal degeneration. If this misreporting occurred differentially depending on fungicide use, the association between retinal degeneration and diabetes that we observed may have been subject to bias. We are not aware of a realistic scenario that would result in such differential misreporting. Furthermore, an association between fungicide use and retinal degeneration persisted when we restricted the analysis to women without a history of diabetes, although the odds ratio was reduced (OR = 1.6, 95 percent CI: 0.9, 2.8).

Diabetic retinopathy, a common complication of diabetes, is a progressive disorder beginning with small aneurysms in the retinal microvasculature, followed by blockage and small hemorrhages, areas of ischemia, and subsequent neovascularization (20). If the effect modification by diabetes that we observed is real, the apparent potentiation of the risk of retinal degeneration from fungicide exposure in diabetics may result from the added insult of the fungicide to an already weakened diabetic retina. A tissue at risk for ischemia may be more susceptible to a variety of chemical agents, such as those that act to disrupt energy metabolism. Alternatively, rats fed excess dietary sugar in the form of high-fructose corn syrup showed more severe symptoms of cholinergic toxicity after treatment with parathion than did rats fed a control diet (21). This observation is not directly related to the current finding of higher risks of retinal degeneration from fungicide exposure in diabetics, but together these findings suggest a possible influence of hyperglycemia on the expression of pesticide toxicity.

The specific fungicides that appeared to be driving the association of the fungicide functional class with retinal degeneration were maneb (or mancozeb) and ziram. These estimates were elevated in the hierarchical regression, although they were not statistically significant. Other specific pesticides that were associated with retinal degeneration were diazinon, imazethapyr, lindane, and aldrin. Dose-response relationships were observed for maneb, ziram, diazinon, and lindane among the farmer pesticide applicators in a previous investigation (12).

Among fungicides identified here as increasing the risk of retinal degeneration were three dithiocarbamate compounds: maneb, mancozeb, and ziram. Much of the knowledge about the toxicity of this class of substances derives from the study of disulfiram (Antabuse; Wyeth Pharmaceuticals, Collegeville, Pennsylvania), a drug widely used to treat alcoholism. Disulfiram interferes with the breakdown of acetaldehyde, a normal metabolite of ethanol, by inhibiting aldehyde dehydrogenase. This inhibition causes high blood concentrations of acetaldehyde when alcohol is consumed along with disulfiram. Ocular complications have been reported in users of disulfiram, both among those still consuming alcohol and among those not consuming alcohol (22). Several herbicidal thiocarbamates also inhibit aldehyde dehydrogenase, leading to a potential sensitivity to alcohol among agriculturally exposed workers (23). Other mechanisms of dithiocarbamate toxicity are also possible. Disulfiram and other thiocarbamates are metabolized to carbon disulfide, a compound that produces degeneration of neuronal axons, including those in the optic nerve and tract (24, 25). However, recent evidence indicates that *N*,*N*-diethyldithiocarbamate, not carbon disulfide or other metabolites, is the proximal metabolite of disulfiram that produces peripheral nerve demyelination (26–28). Neuronal axons located in the retina are unmyelinated, so the relevance of this mechanism to the cases of retinal degeneration reported here is unclear. Indeed, the unknown nature of the pathology underlying our self-reported cases of retinal degeneration makes it difficult to speculate further about the possible toxic mechanisms involved.

Some farm wives in our population reported using many different pesticides during their lifetimes. We chose analytical methods that allowed us to account for the statistical correlation between individual pesticides and which have been proposed to reduce the number of false-positive results when a large number of comparisons are made (29). In addition, we limited the number of statistical tests performed to those based on hypotheses established a priori.

Our case definition may have amalgamated several conditions (i.e., peripheral retinal and macular degeneration) with differing etiologies, thus limiting our ability to detect associations with pesticides. Since recall and reporting accuracy may decrease with age and retinal degeneration is a disease that affects older persons, an age-related bias may have influenced our results. However, the fungicide effect persisted in analyses restricted to wives under 57 years of age. In general, farmers have been shown to provide accurate information with respect to pesticide use and duration (30, 31), as well as lifestyle and agricultural factors (32).

We observed an association of fungicide use with retinal or macular degeneration among wives of farmer pesticide applicators enrolled in the Agricultural Health Study. Major strengths of this study include its large sample size—the largest sample of farm women to date—and the breadth of data collected from participants, allowing for a detailed examination of pesticide use, eye disorders, and potential confounders. Although we were not able to evaluate dose-response relationships between pesticide use and retinal degeneration to help establish causality, we believe our findings lend weight to the similar results previously reported by Kamel et al. (12). Our use of hierarchical models allowed us to analyze multiple pesticides simultaneously while adjusting estimates to improve precision so we could pinpoint specific pesticides that may be associated with retinal degeneration. However, our findings must be considered exploratory, because this study was cross-sectional in design, disease status and pesticide use were self-reported, and our exposure data were limited to self-reported ever use of pesticides.

ACKNOWLEDGMENTS

This work was supported by intramural research funds from the National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services.

The authors are grateful to S. Long for programming assistance and to Drs. A. Geller and M. Longnecker for their comments on an earlier version of this article.

This manuscript was reviewed by the National Health and Environmental Effects Research Laboratory, Office of Research and Development, Environmental Protection Agency, and approved for publication. The mention of trade names and commercial products does not constitute endorsement or recommendation for use.

REFERENCES

1. Pauleikhoff D, Koch JM. Prevalence of age-related macular degeneration. Curr Opin Ophthalmol 1995;6:51–6.
2. van Leeuwen R, Klaver CC, Vingerling JR, et al. Epidemiology of age-related maculopathy: a review. Eur J Epidemiol 2003;18:845–54.
3. Klein R, Peto T, Bird A, et al. The epidemiology of age-related macular degeneration. Am J Epidemiol 2004;137:486–95.
4. Dementi B. Ocular effects of organophosphates: a historical perspective of Saku disease. J Appl Toxicol 1994;14:119–29.
5. Ishikawa S. Chronic optico-neuropathy due to environmental exposure of organophosphate pesticides (Saku disease): clinical and experimental study. Nippon Ganka Gakkai Zasshi 1973;77:1835–86.
6. Misra UK, Nag D, Misra NK, et al. Some observations on the macula of pesticide workers. Hum Toxicol 1985;4:135–45.
7. Boyes WK, Tandon P, Barone S, et al. Effects of organophosphates on the visual system of rats. J Appl Toxicol 1994;13:135–43.
8. Morse DL, Baker EL Jr, Kimbrough RD, et al. Propanil-chloracne and methomyl toxicity in workers of a pesticide manufacturing plant. Clin Toxicol 1979;15:13–21.
9. Taylor JR, Selhorst JB, Houff SA, et al. Chlordecone intoxication in man: clinical observations. Neurology 1978;28:626–30.
10. Maita K, Tsuda S, Shirasu Y. Chronic toxicity studies with thiram in Wistar rats and beagle dogs. Fundam Appl Toxicol 1991;16:667–86.
11. Hellman B, Laryea D. Inhibitory action of benzimidazole fungicides on the in vivo incorporation of [^{3}H]thymidine in various organs of the mouse. Food Chem Toxicol 1990;28:701–6.
12. Kamel F, Boyes WK, Gladen BC, et al. Retinal degeneration in licensed pesticide applicators. Am J Ind Med 2000;37:618–28.
13. Kirrane EF, Hoppin JA, Umbach DM, et al. Patterns of pesticide use and their determinants among wives of farmer pesticide applicators in the Agricultural Health Study. J Occup Environ Med 2004;46:856–65.
14. Gladen BC, Sandler DP, Zahm SH, et al. Exposure opportunities of families of farmer pesticide applicators. Am J Ind Med 1998;34:581–7.
15. Alavanja M, Sandler DP, McMaster SB, et al. The Agricultural Health Study. Environ Health Perspect 1996;104:362–9.

16. Witte JS, Greenland S, Kim L, et al. Multilevel modeling in epidemiology with GLIMMIX. Epidemiology 2000;11:684–8.
17. Vingerling JR, Klaver CC, Hofman A, et al. Epidemiology of age-related maculopathy. Epidemiol Rev 1995;17:347–60.
18. Lewis H. Peripheral retinal degenerations and the risk of retinal detachment. Am J Ophthalmol 2003;136:155–60.
19. Vingerling JR, Dielmans I, Bots ML, et al. Age-related macular degeneration is associated with atherosclerosis: The Rotterdam Study. Am J Epidemiol 1995;142:404–9.
20. Frank R. Diabetic retinopathy. N Engl J Med 2004;350:48–58.
21. Lui J, Karanth S, Pope C. Dietary sugar-induced modulation of parathion toxicity in juvenile and adult rats. (Abstract 1358). Presented at the annual meeting of the Society of Toxicology, Baltimore, Maryland, March 21–25, 2004.
22. Grant WM. Toxicology of the eye. 3rd ed. Springfield, IL: Charles C Thomas, 1986.
23. Quistad GB, Sparks SE, Casida JE. Aldehyde dehydrogenase of mice inhibited by thiocarbamate herbicides. Life Sci 1994;55:1537–44.
24. Eskin TA, Merigan WH, Woods RW. Carbon disulfide effects on the visual system. II. Retinogeniculate degeneration. Invest Ophthalmol Vis Sci 1988;29:519–27.
25. Merigan WH, Woods RW, Zehl D, et al. Carbon disulfide effects on the visual system. I. Visual thresholds and ophthalmoscopy. Invest Ophthalmol Vis Sci 1988;29:512–18.
26. Tonkin EG, Valentine HL, Milatovic DM, et al. *N*,*N*-diethyldithiocarbamate produces copper accumulation, lipid peroxidation and myelin injury in rat periphery nerve. Toxicol Sci 2004;81:160–71. (Advance access electronic publication June 8, 2004).
27. Tonkin EG, Erve JC, Valentine HL. Disulfiram produces a non-carbon disulfide-dependent schwannopathy in the rat. J Neuropathol Exp Neurol 2000;59:786–97.
28. Tonkin EG, Valentine HL, Zimmerman LS, et al. Parenteral *N*,*N*-diethyldithiocarbamate produces segmental demyelination in the rat that is not dependent on cysteine carbamylation. Toxicol Appl Pharmacol 2003;189:139–50.
29. Steenland K, Bray I, Greenland S, et al. Empirical Bayes adjustments for multiple results in hypothesis-generating or surveillance data. Cancer Epidemiol Biomarkers Prev 2000;9:895–903.
30. Blair A, Zahm S. Patterns of pesticide use among farmers: implications for epidemiologic research. Epidemiology 1993;4:55–62.
31. Hoppin JA, Yucel F, Dosemeci M, et al. Accuracy of self-reported pesticide use duration information from licensed pesticide applicators in the Agricultural Health Study. J Expos Anal Environ Epidemiol 2002;12:313–18.
32. Blair A, Tarone R, Sandler DP, et al. Reliability of reporting on lifestyle and agricultural factors by a sample of participants in the Agricultural Health Study from Iowa. Epidemiology 2002;13:94–9.