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Insecticide Use and Breast Cancer Risk among Farmers' Wives in the Agricultural Health Study

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Background: Some epidemiologic and laboratory studies suggest that insecticides are related to increased breast cancer risk, but the evidence is inconsistent. Women engaged in agricultural work or who reside in agricultural areas may experience appreciable exposures to a wide range of insecticides.

Objective: We examined associations between insecticide use and breast cancer incidence among wives of pesticide applicators (farmers) in the prospective Agricultural Health Study.

Methods: Farmers and their wives provided information on insecticide use, demographics, and reproductive history at enrollment in 1993–1997 and in 5-y follow-up interviews. Cancer incidence was determined via cancer registries. Among 30,594 wives with no history of breast cancer before enrollment, we examined breast cancer risk in relation to the women's and their husbands' insecticide use using Cox proportional hazards regression to estimate adjusted hazard ratios (HRs) and 95% confidence intervals (CIs).

Results: During an average 14.7-y follow-up, 39% of the women reported ever using insecticides, and 1,081 were diagnosed with breast cancer. Although ever use of insecticides overall was not associated with breast cancer risk, risk was elevated among women who had ever used the organophosphates chlorpyrifos [HR = 1.4 (95% CI: 1.0, 2.0)] or terbufos [HR = 1.5 (95% CI: 1.0, 2.1)], with nonsignificantly increased risks for coumaphos [HR = 1.5 (95% CI: 0.9, 2.5)] and heptachlor [HR = 1.5 (95% CI: 0.7, 2.9)]. Risk in relation to the wives' use was associated primarily with premenopausal breast cancer. We found little evidence of differential risk by tumor estrogen receptor status. Among women who did not apply pesticides, the husband's use of fonofos was associated with elevated risk, although no exposure–response trend was observed.

Conclusion: Use of several organophosphate insecticides was associated with elevated breast cancer risk. However, associations for the women's and husbands' use of these insecticides showed limited concordance. Ongoing cohort follow-up may help clarify the relationship, if any, between individual insecticide exposures and breast cancer risk. https://doi.org/10.1289/EHP1295

Introduction

Established environmental and genetic risk factors for breast cancer do not explain a large proportion of the ~247,000 cases diagnosed annually in the United States (Siegel et al. 2016), nor the nearly 1.7 million cases diagnosed annually worldwide (Torre et al. 2015). Identifying more of the environmental risk factors for this disease could facilitate interventions to reduce the disease burden.

Pesticides have received particular attention in relation to breast cancer risk because of their ubiquity and because of the ability of certain pesticides to induce mammary tumors in animal models or to cause *in vitro* effects that may be related to breast cancer etiology (Rudel et al. 2007). The apparent endocrine-disrupting effects of some pesticides have raised particular concerns because of the hormonal nature of many known risk factors for breast cancer. Much research on pesticides and breast cancer has focused specifically on organochlorine insecticides because, in part, of their endocrine-disrupting activity; however, various classes of less-studied insecticides and other pesticides also exhibit such activity (McKinlay et al. 2008).

In 2007, 93 million pounds (42 million kg) of insecticide active ingredient were used in the United States, and 892 million pounds (405 kg) were used worldwide (U.S. EPA 2011). The public is exposed to insecticides and other pesticides, generally at low levels (CDC 2009), through the widespread use of these chemicals in agriculture and through their use in homes, yards, and public spaces. Women who are engaged in agricultural work or who reside in agricultural areas are likely to experience higher exposures to a greater range of pesticides. Such agricultural exposures can be direct, resulting from a woman's handling of pesticides (i.e., mixing, applying, or both), or they can be indirect, resulting from working in fields containing pesticide residues. Other indirect pesticide exposures may result from spray drift, contaminated drinking water, or handling of items contaminated in or near areas of pesticide application.

Most epidemiologic studies of pesticide exposure and breast cancer risk among agriculturally exposed women have relied on nonspecific indicators of exposure, such as possession of a pesticide application license, job title, or residence on a farm (Band et al. 2000; Brophy et al. 2012; Fleming et al. 1999; Folsom et al. 1996; Franceschi et al. 1993; Hansen et al. 1992; Kristensen et al. 1996; Pukkala and Notkola 1997; Salerno et al. 2016; Wiklund and Dich 1994). Results from these studies have been mixed, with reports of increased risk (Band et al. 2000; Brophy et al. 2012; Salerno et al. 2016), no association (Blair et al. 1993; Folsom et al. 1996; Hansen et al. 1992), or decreased risk (Fleming et al. 1999; Franceschi et al. 1993; Kristensen et al.

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1996; Pukkala and Notkola 1997; Wiklund and Dich 1994). Two case–control studies based on self-reported pesticide use and agricultural tasks among women in agricultural areas observed decreased risks of breast cancer associated with farming/agricultural work (Duell et al. 2000; Settimi et al. 1999), although one study (Duell et al. 2000) reported increased risk among women with the greatest likelihood of pesticide exposure; however, neither study presented pesticide-specific results. In contrast, a case–control study among Hispanic female farm workers observed an increased risk of breast cancer in relation to chlordane, malathion, and 2,4-dichlorophenoxyacetic acid (2,4-D) exposure (Mills and Yang 2005).

Epidemiologic studies of dichlorodiphenyltrichloroethane (DDT) and other organochlorine insecticides in relation to breast cancer, conducted primarily in the general population, have been largely null (Ingber et al. 2013; Khanjani et al. 2007), although timing of exposure may be important (Cohn et al. 2007; Fenton and Birnbaum 2015). Few studies have examined nonorganochlorine insecticide use and breast cancer risk among the general population, and the results have been inconsistent, with one study observing increased risk (Teitelbaum et al. 2007) and two studies observing no association (El-Zaemey et al. 2014; Farooq et al. 2010); importantly, these studies lacked data to investigate specific pesticides.

The present study follows up on a previous investigation of pesticide use/exposure and breast cancer risk among wives of farmers in the large, prospective Agricultural Health Study (AHS) cohort (Engel et al. 2005). It includes an additional 10–11 y of follow-up and substantially more incident cases. Although our earlier study found several suggestive associations, the relatively short follow-up duration and the modest number of cases limited interpretation of the results. However, the AHS remains one of the only studies with the necessary size and exposure data to examine individual pesticides in relation to breast cancer risk. Because of the extent and complexity of the exposure data, this paper presents risk estimates for a range of insecticides, and a separate paper will present findings for herbicides, fungicides, and fumigants.

Methods

Study Population

The Agricultural Health Study has been described in detail elsewhere (Alavanja et al. 1996). In brief, 52,394 private pesticide applicators, primarily farmers, in Iowa and North Carolina were enrolled in the cohort between 1993 and 1997 while attending mandatory certification sessions for applying restricted-use pesticides. Male private applicators who indicated that they were married were asked to have their wives complete two take-home questionnaires: an enrollment questionnaire focused on the wives' farm exposures and general health and a questionnaire focused on the wives' reproductive health history. A total of 32,126 wives (an estimated 75% of those eligible) enrolled in the cohort. Of these, 19,578 (61% of those enrolled) completed both questionnaires, and 12,548 (39% of those enrolled) completed only the enrollment questionnaire. In addition, 23,676 wives (74%) completed a 5-y follow-up telephone interview (Figure 1).

Exposure Assessment

Pesticide exposure information was obtained at enrollment and at the 5-y follow-up interview. At enrollment, the spouses were asked about ever/never use of 50 specific pesticides, including 22 insecticides. In the 5-y follow-up interview, they were asked whether they had used specific pesticides in the previous growing season and, if so, how many days per week they had typically

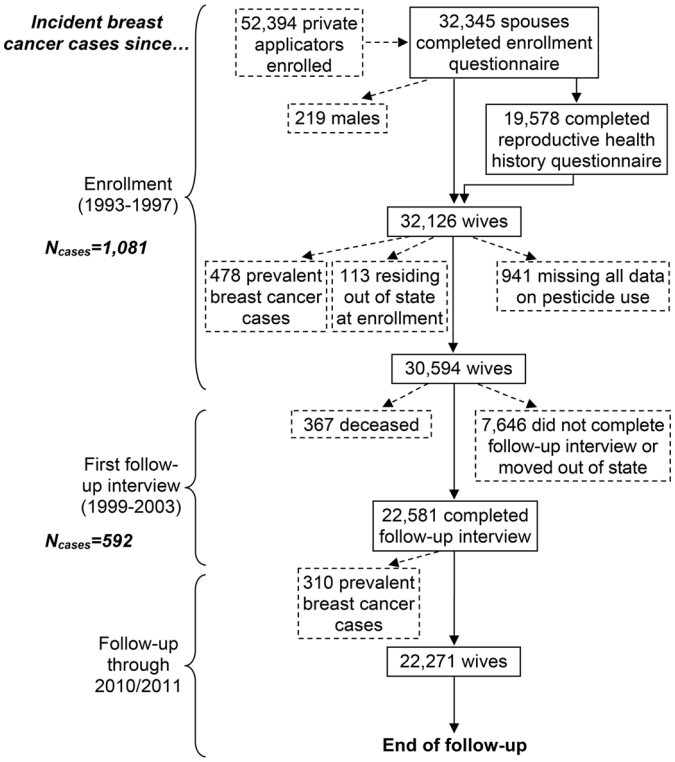

Figure 1. Flow diagram showing the study population for an analysis of insecticide use and incident breast cancer in wives enrolled in the Agricultural Health Study. Boxes with solid lines represent individuals who remained in the analysis after each step, and boxes with dashed lines represent individuals who were excluded after each step. Cancer and mortality follow-up was via registry linkage.

used them. They were also asked detailed questions about their use of personal protective equipment and practices when handling pesticides. In addition, the questionnaires at both time periods elicited information on a range of demographic, lifestyle, health, and reproductive factors.

Enrollment questionnaires for the farmers elicited similar, but more detailed, information on lifetime pesticide use, including duration, frequency, and decade of first use of specific pesticides. In the 5-year follow-up interview, farmers were asked similar pesticide-related questions as were the spouses. This information was used to assess possible indirect pesticide exposure by the farmers' wives. (Questionnaires are available at https://www.aghealth.nih.gov/collaboration/questionnaires.html.)

Participant Follow-up and Case Ascertainment

Incident breast cancer cases were ascertained through population-based cancer registries in Iowa and North Carolina, using *International Classification of Diseases for Oncology* (3rd edition) codes C50.0–C50.9 (Fritz et al. 2013). Vital status was ascertained through state death registries and the National Death Index. The average duration of follow-up from enrollment was 14.7 y (through 31 December 2010 in North Carolina and 31 December 2011 in Iowa), with a total duration of 448,204 person-years.

Data Analysis

We used Cox proportional hazards regression to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for associations between several metrics of insecticide exposure, described below, and breast cancer risk, with age as the time scale and with left

truncation at either enrollment or the 5-y follow-up interview, as appropriate. Participants with a breast cancer diagnosis before enrollment or, for certain analyses, before the 5-y follow-up interview, were excluded. The outcome of interest was first primary invasive breast cancer, with censoring at the time of any *in situ* breast cancer diagnosis. In all analyses, person-time was accrued until the earliest of breast cancer diagnosis, movement out of state, death, or end of follow-up. The proportional hazards assumption was evaluated for each exposure by including a time-varying interaction term, exposure × age, in each model.

Three primary exposure metrics were investigated for individual insecticides: 1) ever/never use by the women, reported at enrollment; 2) total intensity-weighted days of use in the previous growing season by the women, reported in the 5-y follow-up interview; and 3) cumulative potential exposure (days) from the husband's use across the enrollment and follow-up interviews. Metrics 1 and 2 represent direct exposures, and metric 3 represents indirect exposures. We present results for the 20 insecticides that were included in the enrollment questionnaires and that were reported by ≥5 cases in analyses of direct or indirect exposure.

For analyses of ever/never use by the women, we examined risk associated with participants' ever use, reported at enrollment, of each insecticide among the full analytic cohort of 30,594 women.

For analyses of total intensity-weighted days of use in the previous growing season by the women, which took into account the use of personal protective equipment and practices, we used data reported by the women in the 5-y follow-up interview. This analysis was restricted to the 22,271 wives who completed the 5-y interview, had no history of breast cancer prior to this interview, and were living in-state at the time of the interview. Exposure was estimated as intensity score × days of use of each pesticide in the prior growing season. The intensity score was derived from an algorithm that incorporates exposure measurement data from the literature and from the Pesticide Handlers Exposure Database (U.S. EPA 1995), together with self-reported data from each study participant on pesticide use and practices, including whether they mixed specific pesticides, the methods of application used, and the use of personal protective equipment (Coble et al. 2011). Pesticide-specific quantiles of exposure were determined using cut-points from the exposure distribution among noncases, with a minimum of 20 exposed cases per quantile; an exposure was treated as any/none if there were <20 exposed cases.

We examined the risk associated with cumulative potential lifetime exposure to each insecticide from the husband's use among the 13,500 wives who reported no personal pesticide use before enrollment. Exposure was estimated as the average number of days per year that each pesticide was used × the number of years the pesticide was used after marriage. For wives missing data on the year of marriage (38.3%), we assumed the later of the year that the wife or husband was 20 y old. Exposure to each insecticide was assumed to begin at the latest of the husband's reported decade of first use of the insecticide or the year of marriage. Exposure to each insecticide ended at the earliest of the husband's self-reported last use of the insecticide, breast cancer diagnosis, censoring date, or end of follow-up. Insecticide-specific quantiles of exposure were defined as described above. Wives who reported first pesticide use at the 5-y interview or whose pesticide use at the 5-y interview could not be determined were censored at the midpoint between their date of enrollment and their 5-y interview (or the imputed date of the 5-y interview if they did not complete the interview). We used multiple imputation ($n = 5$) to estimate use of individual pesticides at 5 y among the 37% of farmers who did not complete the 5-y interview (Heltshe et al. 2012).

All analyses were adjusted for time-varying menopausal status, race (white, other), state (Iowa, North Carolina), and combined parity/age at first birth (1 birth by 30 y old; ≥2 births, first by 30 y old; nulliparous or all births after 30 y old), with nulliparous women and those with first births after they were 30 y old combined because of the relatively small number of nulliparous cases ($n = 21$ in analyses of indirect exposures). We also adjusted for all other pesticides (including noninsecticides) found to be associated with breast cancer in the present analysis with a demographics-adjusted HR ≥1.50 or ≤0.67 and a minimum of five exposed cases. The set of adjustment pesticides was the same for all analyses within each exposure metric (e.g., for all analyses of ever/never use by the women reported at enrollment) but could vary across exposure metrics, as indicated in the table footnotes. Body mass index, age at menarche, family history of breast cancer, physical activity, cigarette smoking, alcohol consumption, education, usual daily sun exposure, and nonfarm employment were examined as potential confounders but were not included in the final models because they did not materially change risk estimates.

We performed additional analyses examining breast cancer risk associated with the relative extent of the women's use of each insecticide by modeling, in a time-varying manner, whether the women reported its use at *a*) enrollment only, *b*) the 5-y follow-up interview only, *c*) enrollment and the follow-up interview, or *d*) neither. To account for likelihood of exposure (vs. use), we also examined breast cancer risk associated with each insecticide in models among the full analytic cohort that included, in a time-varying manner, both the husband's use and the wife's use of a given insecticide; exposure to each insecticide was defined as *a*) ever use by the husband only, *b*) ever use by the wife (regardless of husband's use), and *c*) neither.

In addition, we conducted analyses stratified by state of residence, menopausal status, and tumor receptor status. We stratified analyses by state primarily to investigate consistency of direction, rather than of magnitude, of associations between states. We stratified menopausal analyses using a product interaction term between insecticide exposure and time-varying menopausal status, using median age at menopause in the cohort as a proxy for individual age at menopause when the latter information was missing. We also conducted analyses defining cases by their estrogen receptor (ER) and progesterone receptor (PR) status (positive or negative) using joint proportional hazards models (Xue et al. 2013). We conducted sensitivity analyses for direct exposures (metric 1, above) restricted to *a*) women who reported ever handling pesticides ($n = 17{,}094$), to increase homogeneity for nonpesticide factors, and *b*) women who reported either never handling pesticides or handling pesticides for a minimum of 10 y ($n = 23{,}431$), to increase exposure contrasts. In addition, we conducted subanalyses of indirect exposures lagged 5, 10, and 15 y ($n = 13{,}500$) and, separately, excluded the imputed pesticide use data among the farmers who did not complete the 5-y follow-up interview. We conducted subanalyses of both direct (metric 1) and indirect (metric 3) exposures in which we excluded cases diagnosed within the first five years after enrollment ($n = 30{,}269$ and $n = 13{,}339$, respectively). We also performed analyses without adjustment for menopausal status to address concerns that pesticide exposure may affect timing of menopause.

Missing data for covariates were imputed using IVEware (University of Michigan, Ann Arbor, MI). Risk estimates that incorporated imputed data were similar to those that included only observed data, so we present risk estimates based on models incorporating imputed covariate data.

The institutional review boards of participating institutions approved the study, including the use of implied informed consent for enrollment. We performed all analyses using SAS (version 9.4; SAS Institute Inc.). Analyses were based on AHS data releases P1REL0906.00, P1REL201209.00, and P2REL201209.00.

Results

In this analytic cohort of 30,594 women, 1,081 women were diagnosed with incident breast cancer during the follow-up period (Table 1). The median age at enrollment was 46 y. Over 98% of the women were white. Most (68.3%) were from Iowa. A majority (79.8%) reported one or more births. Nearly half (46.3%) were premenopausal, and nearly half (44.9%) were overweight or obese at baseline. Characteristics of the subgroup of spouses who never used pesticides were similar to those of the full cohort, although the women who never used pesticides were somewhat more likely to be from North Carolina. Estrogen receptor (ER) and progesterone receptor (PR) status was known for $\sim 83\%$ of cases.

Ever personally using (i.e., mixing or applying) any insecticide was reported by 39.0% of the women and was not associated with risk of breast cancer [HR = 1.0 (95% CI: 0.9, 1.2)] (Table 2). There was no clear evidence of altered risk associated with ever use of any of the insecticide chemical classes examined, including carbamates, organochlorines, and organophosphates. We observed significant associations between breast cancer risk and ever use of chlorpyrifos [HR = 1.4 (95% CI: 1.0, 2.0)] and terbufos [HR = 1.5 (95% CI: 1.0, 2.1)]. Risk was nonsignificantly elevated in relation to ever use of coumaphos [HR = 1.5 (95% CI: 0.9, 2.5)] and heptachlor [HR = 1.5 (95% CI: 0.7, 2.9)] and was nonsignificantly reduced in relation to ever use of toxaphene [HR = 0.6 (95% CI: 0.3, 1.5)]. Risk estimates adjusted only for demographic/reproductive factors were similar to those additionally adjusted for other pesticides associated with breast cancer in the present analysis, except for heptachlor and terbufos, whose risk estimates increased appreciably in the more fully adjusted models. Risk estimates for chlorpyrifos and terbufos decreased slightly when mutually adjusted for [HR = 1.3 (95% CI: 0.9, 1.9) and HR = 1.3 (95% CI: 0.9, 2.0), respectively].

We observed a nonsignificantly elevated risk of breast cancer associated with intensity-weighted days of diazinon use reported by the women at the 5-y follow-up interview [HR = 1.4 (95% CI: 0.9, 2.1)] (Table 3). Risk estimates were similar when we adjusted for only demographic factors (i.e., reducing possible overfitting of models). However, analyses at the 5-y follow-up were limited by the relatively small number of women reporting use of insecticides at this interview, which precluded analysis of most insecticides. Moreover, the use of certain insecticides was sufficiently rare to permit comparisons only of exposed versus unexposed.

The highest tertile of fonofos use by the husbands was associated with a significantly elevated breast cancer risk of 1.7 (95% CI: 1.0, 2.7) among the women who did not apply pesticides themselves; however, there was no apparent exposure–response trend ($p = 0.09$) (Table 4). Ever use of malathion by the husband was associated with a reduced risk of 0.7 (95% CI: 0.5, 1.0) but also with no apparent exposure–response trend ($p = 0.23$). Risk was nonsignificantly elevated in relation to the highest quartile of terbufos use by the husband [HR = 1.4 (95% CI: 0.9, 2.2)]. Interpretation of the results did not change in sensitivity analyses that excluded imputed 5-y insecticide use data for the husbands (data not shown).

In analyses examining the relative extent of the women's insecticide use, the risk associated with diazinon appeared to be stronger among women who reported its use at both enrollment and the 5-y follow-up [HR = 1.9 (95% CI: 0.9, 4.1)] than at either enrollment [HR = 1.1 (95% CI: 0.8, 1.5)] or the 5-y follow-up [HR = 1.5 (95% CI: 0.8, 3.0)] alone (see Table S1). Risk was significantly elevated among women who reported use of malathion at both enrollment and the 5-y follow-up [HR = 1.9 (95% CI: 1.1, 3.2)], but there were too few women who reported its use only at follow-up to estimate risk in this group.

In analyses that included ever use of insecticides by both the women and their husbands, risk was increased in relation to the women's use of phorate [HR = 2.0 (95% CI: 1.0, 4.1)], carbofuran [HR = 1.7 (95% CI: 0.7, 4.4)], chlorpyrifos [HR = 1.6 (95% CI: 0.9, 2.9)], and terbufos [HR = 1.7 (95% CI: 0.9, 3.5)], although only the first was statistically significant (see Table S2). Risk was reduced in relation to the husbands' use of DDT [HR = 0.5 (95% CI: 0.3, 0.8)] and phorate [HR = 0.7 (95% CI: 0.4, 0.9)].

The results were generally similar between states for insecticides with sufficient numbers of exposed to permit stratified analyses. For example, ever use of chlorpyrifos by the women was associated with similarly increased risks in Iowa [HR = 1.5 (95% CI: 1.0, 2.2)] and North Carolina [HR = 1.4 (95% CI: 0.8, 2.5)] (see Table S3). However, ever use of diazinon by the women was associated with an increased risk only in North Carolina [HR = 1.4 (95% CI: 1.0, 1.9)] and not in Iowa [HR = 0.9 (95% CI: 0.7, 1.2)]. Ever use of terbufos by the women was more strongly associated with risk in North Carolina [HR = 2.5 (95% CI: 1.1, 5.5)] than in Iowa [HR = 1.3 (95% CI: 0.9, 2.1)]. Ever use of lindane by the husband was associated with an elevated risk in North Carolina [HR = 1.8 (95% CI: 0.9, 3.8)] but not in Iowa [HR = 0.8 (95% CI: 0.5, 1.5)] (see Table S6). We observed a decreased risk associated with ever use of carbaryl and DDT by the husband in North Carolina [HR = 0.5 (95% CI: 0.3, 1.0) and HR = 0.5 (95% CI: 0.2, 1.0), respectively], but no such inverse association was observed in Iowa [HR = 0.9 (95% CI: 0.6, 1.4) and HR = 1.2 (95% CI: 0.7, 2.1), respectively].

Ever use of several insecticides by the women was associated with only premenopausal breast cancer, although the number of exposed premenopausal cases in most analyses was small (see Table S4). These included phorate [premenopausal HR = 2.5 (95% CI: 1.0, 6.2); postmenopausal HR = 0.9 (95% CI: 0.5, 1.6); $p_{\text{interaction}} = 0.05$] and terbufos [premenopausal HR = 2.6 (95% CI: 1.3, 5.4); postmenopausal HR = 1.2 (95% CI: 0.8, 1.9); $p_{\text{interaction}} = 0.08$], with weaker evidence for dichlorvos ($p_{\text{interaction}} = 0.15$). Chlorpyrifos was associated with an increased risk of premenopausal breast cancer [HR = 1.9 (95% CI: 1.0, 3.8)] and with a weaker, nonsignificantly increased risk of postmenopausal breast cancer [HR = 1.3 (95% CI: 0.9, 1.9)]. Associations with ever use of fonofos by the husband appeared to differ by menopausal status [premenopausal HR = 0.6 (95% CI: 0.3, 1.4); postmenopausal HR = 1.5 (95% CI: 1.1, 2.2); $p_{\text{interaction}} = 0.04$] (see Table S7).

In analyses stratified by tumor ER status, we observed no important differences in risk related to the women's use of insecticides, but we found elevated risk associated with the husbands' ever use of DDT only for ER– tumors [ER+ HR = 0.8 (95% CI: 0.5, 1.4); ER– HR = 1.9 (95% CI:0.7, 4.9); $p_{\text{interaction}} = 0.10$] (see Tables S5 and S8). However, the low incidence of ER– tumors in this cohort limited our ability to identify such differences.

When we excluded cases diagnosed within the first five years after enrollment, risk estimates became stronger in relation to the women's ever use of organophosphates [HR = 1.3 (95% CI: 1.0, 1.5)] and parathion [1.9 (95% CI: 1.0, 3.4)] and to the husbands' ever use of carbaryl [HR = 0.6 (95% CI: 0.4, 1.0)]. Risk estimates were similar when we lagged the husbands' insecticide use by up to 15 y, when we examined associations by joint ER+/PR+ status

Table 1. Selected characteristics at enrollment (unless otherwise noted) of farmers' wives in the Agricultural Health Study.

	All wives in the cohort				Wives who never used pesticides			
	Cases ($n = 1,081$)		Noncases ($n = 29,513$)		Cases ($n = 376$)		Noncases ($n = 13,124$)	
Characteristic	*n*	%	*n*	%	*n*	%	*n*	%
Age (y)								
18–39	142	13.1	9,410	31.9	43	11.4	4,594	35.0
40–49	293	27.1	8,465	28.7	101	26.9	3,371	25.7
50–59	377	34.9	6,861	23.2	126	33.5	2,738	20.9
60–69	214	19.8	3,848	13.0	84	22.3	1,874	14.3
70–91	55	5.1	929	3.1	22	5.9	547	4.2
Race								
White	1,059	98.0	28,948	98.1	367	97.6	12,709	96.8
Other	21	1.9	514	1.7	9	2.4	386	2.9
Missing	1	0.1	51	0.2	0	0.0	29	0.2
State of residence								
Iowa	703	65.0	20,182	68.4	224	59.6	8,121	61.9
North Carolina	378	35.0	9,331	31.6	152	40.4	5,003	38.1
First-degree family history of breast cancer	206	19.1	3,253	11.0	73	19.4	1,393	10.6
Body mass index (kg/m^2)								
<25.0	434	40.1	12,926	43.8	143	38.0	5,708	43.5
25.0–29.9	331	30.6	8,343	28.3	112	29.8	3,536	26.9
≥30.0	217	20.1	4,822	16.3	85	22.6	2,105	16.0
Missing	99	9.2	3,422	11.6	36	9.6	1,775	13.5
Age at menarche (y)								
<12	144	13.3	3,836	13.0	46	12.2	1,604	12.2
12–14	721	66.7	19,071	64.6	253	67.3	8,260	62.9
≥15	98	9.1	2,566	8.7	37	9.8	1,185	9.0
Missing	118	10.9	4,040	13.7	40	10.6	2,075	15.8
Parity								
Nulliparous	48	4.4	1,563	5.3	21	5.6	740	5.6
1	88	8.1	2,189	7.4	37	9.8	1,148	8.7
≥2	823	76.1	21,316	72.2	270	71.8	8,960	68.3
Missing	122	11.3	4,445	15.1	48	12.8	2,276	17.3
Age at first birth (y)[a]								
≤20	255	24.7	6,674	23.9	77	21.7	2,888	23.3
21–30	585	56.6	15,789	56.5	210	59.2	6,706	54.2
>30	71	6.9	1,239	4.4	25	7.0	613	4.9
Missing	122	11.8	4,248	15.2	43	12.1	2,177	17.6
Menopausal status at enrollment								
Premenopausal	385	35.6	14,393	48.8	126	33.5	6,241	47.6
Postmenopausal	584	54.0	11,262	38.2	210	55.9	4,916	37.5
Missing	112	10.4	3,858	13.1	40	10.6	1,967	15.0
Age at menopause (y)[b]								
Premenopausal	201	18.6	9,653	32.7	67	17.8	4,106	31.3
<45	202	18.7	4,728	16.0	56	14.9	2,107	16.1
45–49	171	15.8	3,361	11.4	70	18.6	1,456	11.1
50–54	259	24.0	4,165	14.1	97	25.8	1,688	12.9
≥55	71	6.6	1,211	4.1	23	6.1	482	3.7
Missing	177	16.4	6,395	21.7	63	16.8	3,285	25.0
Highest educational attainment								
Less than high school	62	5.7	1,429	4.8	25	6.6	865	6.6
High school	420	38.9	10,617	36.0	156	41.5	5,005	38.1
More than high school	504	46.6	14,346	48.6	166	44.1	6,192	47.2
Other	91	8.4	3,022	10.2	26	6.9	1,003	7.6
Missing	4	0.4	99	0.3	3	0.8	59	0.4
Smoking status								
Current	88	8.1	3,000	10.2	37	9.8	1,452	11.1
Former	205	19.0	4,943	16.7	69	18.4	2,063	15.7
Never	777	71.9	21,262	72.0	266	70.7	9,440	71.9
Missing	11	1.0	308	1.0	4	1.1	169	1.3
Tumor estrogen/progesterone receptor status								
ER +	712	65.9	NA		243	64.6	NA	
ER −	190	17.6	NA		64	17.0	NA	
Missing	179	16.6	NA		69	18.4	NA	
PR +	619	57.3	NA		216	57.5	NA	
PR −	277	25.6	NA		90	23.9	NA	
Missing	185	17.1	NA		70	18.6	NA	
Lifetime number of insecticides used								
0	627	58.0	17,949	60.8	376	100.0	13,124	100.0
1	178	16.5	4,851	16.4	0	0.0	0	0.0
2	125	11.6	2,906	9.9	0	0.0	0	0.0
≥3	144	13.3	3,661	12.4	0	0.0	0	0.0

[a]Restricted to parous women.
[b]Prior to the earliest of breast cancer diagnosis, censoring, or end of follow-up.

Table 2. Associations between the wives' ever use of individual insecticides at enrollment and risk of breast cancer among farmers' wives in the Agricultural Health Study ($n = 30,594$).

Exposure	Exposed cases ($n = 1,081$)	Exposed noncases ($n = 29,513$)	Adjusted HR[a]	95% CI	Adjusted HR[b]	95% CI
Any insecticide	447	11,420	1.0	0.9, 1.1	1.0	0.9, 1.2
Carbamates	353	9,089	1.0	0.9, 1.1	1.0	0.9, 1.1
Carbaryl	342	8,884	1.0	0.8, 1.1	1.0	0.9, 1.1
Carbofuran	20	535	0.9	0.6, 1.3	1.0	0.6, 1.7
Organochlorines	87	2,149	0.8	0.7, 1.0	0.9	0.7, 1.1
Aldrin	8	232	0.7	0.3, 1.4	0.7	0.3, 1.8
Chlordane	47	1,178	0.8	0.6, 1.1	0.9	0.6, 1.2
DDT	48	997	0.9	0.7, 1.2	0.9	0.6, 1.3
Dieldrin	6	102	1.2	0.5, 2.7	1.3	0.5, 3.6
Heptachlor	11	215	1.1	0.6, 1.9	1.5	0.7, 2.9
Lindane	15	425	0.8	0.5, 1.4	0.9	0.5, 1.6
Toxaphene	6	198	0.6	0.3, 1.4	0.6	0.3, 1.5
Organophosphates	300	7,389	1.0	0.9, 1.2	1.1	0.9, 1.2
Chlorpyrifos	51	1,130	1.2	0.9, 1.6	1.4	1.0, 2.0
Coumaphos	18	356	1.3	0.8, 2.0	1.5	0.9, 2.5
Diazinon	118	2,902	1.1	0.9, 1.3	1.1	0.9, 1.3
Dichlorvos	32	712	1.1	0.8, 1.5	1.1	0.7, 1.6
Fonofos	18	538	0.8	0.5, 1.3	0.9	0.5, 1.7
Malathion	226	5,561	1.0	0.9, 1.2	1.0	0.8, 1.2
Parathion	13	289	1.1	0.7, 1.9	1.3	0.7, 2.4
Phorate	22	561	1.0	0.6, 1.5	1.1	0.7, 1.8
Terbufos	37	814	1.2	0.8, 1.6	1.5	1.0, 2.1
Pyrethroids	45	1,360	0.9	0.7, 1.2	0.9	0.6, 1.3
Permethrin for animals	32	977	0.9	0.7, 1.3	0.8	0.6, 1.3
Permethrin for crops	17	568	0.8	0.5, 1.3	0.8	0.5, 1.5

Note: CI, confidence interval; DDT, dichlorodiphenyltrichloroethane; HR, hazard ratio.
[a]Time scale is attained age, with left truncation at enrollment. Adjusted for time-varying menopausal status, race, state, and combined parity/age at first birth.
[b]Adjusted as in ([a]) and additionally adjusted for use of benomyl, metribuzin, butylate, and toxaphene, except for "Any insecticide" and "Organochlorines," which were additionally adjusted for use of benomyl, metribuzin, and butylate only.

rather than by ER+ status alone, and when we did not adjust for menopausal status (data not shown).

When we simultaneously included in a model all significantly associated insecticide exposures (i.e., direct and indirect) among the full cohort, the HRs associated with the wives' ever use of chlorpyrifos and terbufos were 1.8 (95% CI: 1.0, 3.3) and 1.4 (95% CI: 0.7, 2.8), respectively, and those associated with the husbands' ever use of fonofos and malathion were 1.2 (95% CI: 0.9, 1.6) and 0.8 (95% CI: 0.6, 1.0), respectively.

Discussion

The results from this large, prospective cohort study of women living or working on farms, or both, suggest that certain organophosphate insecticides may be associated with elevated risk of breast cancer. We observed modestly increased risks associated with the women's use of the organophosphates chlorpyrifos and terbufos, with more limited evidence for coumaphos, diazinon, and heptachlor. However, there was little consistency between associations for direct and indirect exposures. None of the insecticides that were associated with increased breast cancer risk when used by the wives were significantly associated with increased risk when used by the husbands. Terbufos showed a nonsignificantly elevated risk in relation to the husbands' use, and fonofos, which was not associated with risk in relation to the wives' use, showed a significantly elevated risk in relation to the highest category of the husband's use. It is noteworthy that chlorpyrifos and terbufos were associated with elevated risk in both

Table 3. Associations between the wives' intensity-weighted days per year of use of individual insecticides at the 5-year follow-up and risk of breast cancer among farmers' wives in the Agricultural Health Study ($n = 22,271$).

Exposure	Level (intensity-weighted days)	Exposed cases ($n = 592$)	Exposed noncases ($n = 21,679$)	Adjusted HR[a]	95% CI	Adjusted HR[b]	95% CI
Chlorpyrifos	None	584	21,464	1		1	
	Any	8	215	1.4	0.7, 2.8	1.4	0.7, 2.8
Diazinon	None	571	21,069	1		1	
	Any	21	610	1.3	0.9, 2.1	1.4	0.9, 2.1
Malathion	None	567	20,896	1		1	
	Any	25	783	1.2	0.8, 1.8	1.2	0.8, 1.8
Carbaryl[c]	None	508	18,346	1		1	
	Any	84	3,333	0.9	0.7, 1.1	0.8	0.7, 1.1
	Quartile 1	20	864	0.8	0.5, 1.3	0.8	0.5, 1.3
	Quartile 2	21	856	0.9	0.6, 1.3	0.8	0.5, 1.3
	Quartile 3	21	822	0.8	0.5, 1.3	0.8	0.5, 1.3
	Quartile 4	22	791	0.9	0.6, 1.4	0.9	0.6, 1.4
				p trend = 0.23		p trend = 0.16	

Note: CI, confidence interval; HR, hazard ratio.
[a]Time scale is attained age, with left truncation at 5-year follow-up interview. Adjusted for time-varying menopausal status, race, state, and combined parity/age at first birth.
[b]Adjusted as in ([a]) and additionally adjusted for use of dicamba, cyfluthrin, *Bacillus thuringiensis*, and mecoprop-p.
[c]Showed evidence of nonproportional hazards ($p = 0.02$–0.03).

Table 4. Associations between the husbands' use of individual insecticides and risk of breast cancer among farmers' wives who never used pesticides in the Agricultural Health Study ($n = 13,500$).

Exposure	Level	Exposed cases ($n = 376$)[a]	Exposed noncases ($n = 13,124$)	Adjusted HR[b]	95% CI	Adjusted HR[c]	95% CI
Ever/never							
Carbamates							
Aldicarb	Never	313	10,858	1		1	
	Ever	10	539	0.6	0.3, 1.1	0.8	0.4, 1.5
Organochlorines							
Aldrin	Never	249	10,186	1		1	
	Ever	50	956	1.2	0.9, 1.7	1.2	0.8, 1.9
Chlordane	Never	238	9,391	1		1	
	Ever	43	1,041	0.9	0.7, 1.3	0.8	0.5, 1.3
Dieldrin	Never	306	11,499	1		1	
	Ever	18	255	1.6	0.9, 2.6	1.2	0.6, 2.3
Heptachlor	Never	268	10,514	1		1	
	Ever	41	762	1.2	0.9, 1.8	1.2	0.7, 1.9
Lindane	Never	270	9,970	1		1	
	Ever	39	785	1.2	0.8, 1.7	1.1	0.7, 1.7
Toxaphene	Never	287	10,632	1		1	
	Ever	27	658	0.9	0.6, 1.4	1.0	0.6, 1.7
Organophosphates							
Coumaphos	Never	305	10,766	1		1	
	Ever	18	743	0.7	0.4, 1.2	0.9	0.5, 1.6
Diazinon	Never	224	8,417	1		1	
	Ever	52	1,307	1.1	0.8, 1.5	1.2	0.8, 1.8
Dichlorvos	Never	291	10,765	1		1	
	Ever	29	917	1.0	0.7, 1.5	0.7	0.4, 1.3
Parathion	Never	283	10,447	1		1	
	Ever	17	440	1.0	0.6, 1.6	1.1	0.6, 2.0
Pyrethroids							
Permethrin for animals	Never	292	10,361	1		1	
	Ever	32	1,440	0.9	0.6, 1.3	0.8	0.5, 1.3
Permethrin for crops	Never	291	10,155	1		1	
	Ever	31	1,415	0.9	0.6, 1.3	0.7	0.4, 1.2
Median							
DDT	None	230	9,296	1		1	
	Any	65	1,345	1.1	0.8, 1.5	0.8	0.5, 1.3
	≤Median	26	505	1.1	0.7, 1.6	0.6	0.3, 1.1
	>Median	24	504	1.0	0.7, 1.6	1.1	0.6, 1.8
				p trend = 0.88		p trend = 0.58	
Phorate	None	223	8,440	1		1	
	Any	58	1,779	0.9	0.7, 1.2	0.8	0.6, 1.2
	≤Median	24	825	0.8	0.5, 1.2	0.8	0.5, 1.3
	>Median	30	845	1.0	0.6, 1.4	0.9	0.5, 1.5
				p trend = 0.49		p trend = 0.53	
Tertiles							
Carbaryl	None	138	5,616	1		1	
	Any	98	2,872	0.9	0.7, 1.2	0.8	0.5, 1.1
	1	27	792	0.9	0.6, 1.3	0.9	0.5, 1.4
	2	26	829	1.0	0.6, 1.6	0.7	0.4, 1.2
	3	25	772	1.0	0.6, 1.5	1.0	0.6, 1.7
				p trend = 0.83		p trend = 0.64	
Carbofuran	None	238	9,091	1		1	
	Any	78	2,385	1.0	0.7, 1.2	1.1	0.8, 1.5
	1	29	910	0.8	0.6, 1.2	0.9	0.5, 1.3
	2	19	636	1.0	0.6, 1.8	1.2	0.6, 2.3
	3	26	764	1.1	0.7, 1.6	1.3	0.8, 2.1
				p trend = 0.79		p trend = 0.57	
Fonofos	None	253	9,633	1		1	
	Any	71	2,142	1.1	0.9, 1.5	1.3	0.9, 1.8
	1	21	696	1.1	0.8, 1.7	1.1	0.7, 1.8
	2	25	758	1.0	0.6, 1.7	1.1	0.6, 2.0
	3	23	640	1.2	0.8, 1.9	1.7	1.0, 2.7
				p trend = 0.36		p trend = 0.09	
Malathion	None	100	3,860	1		1	
	Any	121	3,997	0.8	0.6, 1.1	0.7	0.5, 1.0
	1	31	1,194	0.7	0.5, 1.0	0.6	0.4, 1.0
	2	35	1,178	1.3	0.8, 2.0	1.4	0.8, 2.4
	3	29	1,185	0.8	0.5, 1.1	0.7	0.5, 1.2
				p trend = 0.21		p trend = 0.23	

Table 4 (*Continued.*)

Exposure	Level	Exposed cases ($n = 376$)[a]	Exposed noncases ($n = 13{,}124$)	Adjusted HR[b]	95% CI	Adjusted HR[c]	95% CI
Quartiles							
Chlorpyrifos	None	218	7,611	1		1	
	Any	140	4,864	1.0	0.8, 1.3	1.0	0.8, 1.4
	1	39	1,198	1.1	0.8, 1.5	1.0	0.6, 1.5
	2	42	1,117	1.3	1.0, 1.9	1.5	1.0, 2.2
	3	26	1,116	0.9	0.6, 1.3	0.8	0.4, 1.3
	4	25	1,145	0.9	0.6, 1.3	1.0	0.6, 1.6
				p trend = 0.79		p trend = 0.72	
Terbufos	None	208	7,647	1		1	
	Any	116	4,098	1.0	0.8, 1.3	1.2	0.9, 1.6
	1	30	976	1.1	0.8, 1.7	1.2	0.7, 1.9
	2	20	977	0.7	0.5, 1.2	0.9	0.5, 1.5
	3	29	985	1.0	0.7, 1.5	1.1	0.7, 1.8
	4	31	979	1.1	0.8, 1.6	1.4	0.9, 2.2
				p trend = 0.86		p trend = 0.26	

Note: CI, confidence interval; DDT, dichlorodiphenyltrichloroethane; HR, hazard ratio.
[a]Numbers of exposed and unexposed may not sum to 100% for some insecticides owing to missing data.
[b]Time scale is attained age, with left truncation at enrollment. Adjusted for time-varying menopausal status, race, state, and combined parity/age at first birth.
[c]Adjusted as in ([b]) and additionally adjusted for use of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 2-(2,4,5-trichlorophenoxy)propanoic acid (fenoprop; 2,4,5-TP), trifluralin, aldicarb, and dieldrin, except for "Organochlorines," which was additionally adjusted for 2,4,5-T, 2,4,5-TP, and trifluralin only.

Iowa and North Carolina. Risks associated with the organophosphates phorate, terbufos, and fonofos appeared to vary by menopausal status.

Many of the present findings differ from those of an earlier analysis of this cohort that used pesticide and covariate data only from enrollment and that included only the 309 cases identified during the first 5 y of follow-up (Engel et al. 2005). The present analysis improved upon the earlier analysis by including substantially more cases ($n = 1{,}081$) and follow-up time and by incorporating data on changes in menopausal status over time since enrollment. It is noteworthy that in both studies, increased risks were associated with chlorpyrifos and terbufos and possibly with dichlorvos among premenopausal women. The elevated risks associated with chlorpyrifos and terbufos in the present study are also similar to those observed in a recent study of organophosphate insecticides and cancer incidence among applicators' spouses in the AHS that used only exposure and demographic data collected at enrollment (Lerro et al. 2015).

There is little other epidemiologic research on specific nonpersistent insecticides in relation to breast cancer risk. A small, registry-based case–control study of breast cancer among Hispanic farm workers in California observed increased risk associated with use of the organophosphate malathion and the organochlorine chlordane, although malathion showed no exposure–response trend (Mills and Yang 2005). Another study observed increased risk among farming women most likely to be exposed to pesticides (Duell et al. 2000) but was unable to examine individual pesticide active ingredients.

The International Agency for Research on Cancer (IARC) recently classified parathion as "possibly carcinogenic to humans" (Group 2B) and malathion as "probably carcinogenic to humans" (Group 2A) based, in part, on induction of mammary gland adenocarcinomas in rats following subcutaneous injection (Guyton et al. 2015). In the present study, parathion was not associated with breast cancer risk, and malathion showed only limited and inconsistent evidence of association. In fact, malathion use by the husbands appeared to be associated with a slightly reduced risk. However, another organophosphate, chlorpyrifos, which was associated with increased breast cancer risk in this study, has been observed to act as a potential endocrine disruptor *in vivo* (Ventura et al. 2016) and *in vitro* (Andersen et al. 2002), altering levels of circulating and bioavailable sex hormones (Hodgson and Rose 2006). Chlorpyrifos also induces cell proliferation (Ventura et al. 2016), oxidative stress (Ventura et al. 2015), and genotoxicity (Rahman et al. 2002). However, a rodent bioassay of chlorpyrifos found no evidence of carcinogenicity (Yano et al. 2000). Terbufos and fonofos also alter steroid hormone metabolism (Hodgson and Rose 2006) and induce reactive oxygen species (Hung et al. 2015) and DNA damage (Wu et al. 2011).

The present study did not observe clear associations between any organochlorines and risk of breast cancer, but there was a slight, nonsignificant excess risk with heptachlor. The weight of evidence does not support links of DDT or other organochlorine insecticides with risk of breast cancer (Ingber et al. 2013; Khanjani et al. 2007), although IARC recently classified DDT as "probably carcinogenic to humans" (Group 2A) based on evidence of positive associations with non-Hodgkin lymphoma (NHL), testicular cancer, and liver cancer (Loomis et al. 2015), and limited evidence suggests that early-life exposure to DDT may be important for risk (Fenton and Birnbaum 2015).

The limited evidence in this study that the risks associated with certain pesticides differ by menopausal status, with associations observed primarily among premenopausal cases, may be due to chance or may reflect underlying and yet unclear biological mechanisms. A recent review and meta-analysis concluded that menopausal status did not appear to modify associations between organochlorine insecticides and breast cancer risk (Ingber et al. 2013). We are unaware of any studies that have examined this factor in relation to other insecticides.

This study had several limitations. Some associations may have occurred by chance, given the large number of insecticides investigated. However, we attempted to mitigate this concern by examining the consistency of risk estimates between states and between the wives' and husbands' use, although these are imperfect comparisons because of likely differences in the extent of exposure. In addition, we were unable to assess risk associated with the women's lifetime cumulative use of individual insecticides because, although quantitative use information was collected for the postenrollment period, only ever/never use was assessed for the preenrollment period. Further, despite the large size of the cohort, the limited number of cases exposed to some insecticides precluded exposure–response analyses. We also lacked data on early-life exposures to insecticides, which may be important in breast cancer etiology (Fenton and Birnbaum 2015). These

analyses were also unable to examine the main effects or confounding of so-called "inert" ingredients in pesticide formulations, which can show substantial biological activity (Defarge et al. 2016), but which are protected as trade secrets and only rarely disclosed. These are, however, exposures that are integral to the use of pesticides and not extraneous to it. Finally, we relied on self-reported pesticide use information, which may have resulted in some nondifferential exposure misclassification (Blair et al. 2011). In a prospective cohort study, such misclassification would tend to attenuate risk estimates and flatten exposure–response relationships (Blair et al. 2007). However, the reliability of self-reported ever use of specific pesticides (Blair et al. 2002) and the accuracy of the intensity score (Thomas et al. 2010) have been shown to be high among applicators in this cohort.

This study had several strengths over most previous investigations of insecticides and breast cancer risk. Exposure information was collected before disease diagnosis, mitigating concerns about bias resulting from differential reporting. The large cohort size, together with the wide range of pesticides used by the women or by their husbands, enabled investigation of many individual pesticides. We also had substantially more detailed information on the use of individual pesticides by both the women and their husbands than was available to most previous studies. We had extensive data on potential confounding factors and effect measure modifiers. Lastly, we had excellent follow-up of cohort members over $\sim$15y.

Conclusion

In conclusion, the results from this prospective cohort study suggest that the use of certain organophosphate insecticides, including terbufos, chlorpyrifos, and fonofos, and possibly coumaphos and the organochlorine heptachlor, may be associated with elevated risk of breast cancer. Among these insecticides and in this agricultural population, only terbufos appears to be associated with increased risk in relation to both the women's and their husbands' use, although terbufos and chlorpyrifos were associated with elevated risk in both Iowa and North Carolina. Risk may be greater among premenopausal women. Given the widespread use of these insecticides, further research—and potential replication—of these associations is needed.

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