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Original Contribution

Pesticide Exposure and Timing of Menopause

The Agricultural Health Study

Sherry L. Farr[1,2], Jianwen Cai[3], David A. Savitz[4], Dale P. Sandler[2], Jane A. Hoppin[2], and Glinda S. Cooper[2]

[1] Department of Epidemiology, School of Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC.
[2] National Institute of Environmental Health Sciences, Research Triangle Park, NC.
[3] Department of Biostatistics, School of Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC.
[4] Department of Community and Preventive Medicine, Mount Sinai School of Medicine, New York, NY.

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Age at menopause has implications for fertility and risk of hormonally related chronic diseases. Some pesticides disrupt reproductive hormones or are toxic to the ovary, but little is known about the association between pesticide exposure and timing of menopause. Cox proportional hazards modeling was used to examine the association between use of pesticides and age at menopause among 8,038 women living and working on farms in Iowa and North Carolina. Premenopausal women aged 35–55 years were followed from enrollment (1993–1997) to the date of their last menstrual period, or their follow-up interview (1999–2003) if still premenopausal. Women who experienced surgical menopause were censored at the date of surgery. Approximately 62% of the women reported ever mixing or applying pesticides; women who had never used pesticides were the comparison group for all analyses. After control for age, smoking status, and past use of oral contraceptives, the median time to menopause increased by approximately 3 months for women who used pesticides (hazard ratio = 0.87, 95% confidence interval: 0.78, 0.97) and by approximately 5 months for women who used hormonally active pesticides (hazard ratio = 0.77, 95% confidence interval: 0.65, 0.92). Pesticide use may be associated with a later age at menopause.

agriculture; endocrine disruptors; fertility; hormone antagonists; hormones; menopause; ovary; pesticides

Abbreviations: CI, confidence interval; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; HR, hazard ratio.

The mean age at which natural menopause occurs is 50–52 years in the general population (1, 2). Timing of menopause affects fertility in late reproductive years and may affect a woman's risk of chronic diseases associated with reproductive hormones (3, 4). Ovotoxic and endocrine-disrupting chemicals, such as tobacco smoke (5) and chemotherapy (6), may shorten the time to menopause by accelerating ovarian atresia, increasing the rate of ovulation, or disrupting the hormonal feedback signals affecting follicular development and ovulation. Reproductive factors such as parity and longer menstrual cycles, and possibly use of oral contraceptives, may lead to fewer ovulatory cycles and a later age at menopause (7–9), although these associations and proposed mechanisms are not firmly established. Alcohol consumption may also be associated with a later menopause through mechanisms that are unclear (10).

Relatively few studies in humans have examined the effect of pesticides on ovarian senescence, although experimental studies suggest that specific pesticides may disrupt ovulation or the estrus cycle, reduce the number of ovarian follicles or corpora lutea, or alter hormone levels or receptor binding in various animals (table 1). Atrazine (11–15),

Correspondence to Dr. Sherry L. Farr, 4770 Buford Highway, MS K-34, Atlanta, GA 30341 (e-mail: bwa0@cdc.gov; sherry@alumni.unc.edu).

TABLE 1. Hormonal and ovarian effects of six hormonally active or ovotoxic pesticides* in the Agricultural Health Study, Iowa and North Carolina, 1993–2003

Pesticide	Hormones affected (reference no.)	Effect on the estrus cycle (reference no.)	Effect on the ovary (reference no.)
Atrazine	Antiandrogen: reduced testosterone levels in rats (49–51); progesterone: reduced progesterone levels or progesterone receptor binding in rats (52–55); FSH†/LH† suppression in rats (12, 50, 51, 53)	Extended diestrus with regular cycling in pigs and rats (14, 15, 56, 57); prolonged estrus in rats (13, 54, 58)	Decreased ovarian weight and increased ovarian follicular cysts in rats and pigs (11–15)
Carbaryl	Unknown	Prolonged estrous cycle in rats (16, 18)	Atrophic and necrotic effects on the ovary, disturbed enzymatic activity in the ovary, and inhibited oogenesis in rats (16–21)
Carbon tetrachloride	Thyroid: decreased thyroid hormones thyroxine (T4) and triiodothyronine (T3) in rats (34, 59–61); progesterone: increased progesterone levels in exposed rats (30–33)	Unknown	Decreased ovarian weight and lack of healthy follicles in treated rats (62, 63)
DDT† (and related compounds)‡	Estrogen: estrogen receptor binding, increased uterine weight, precocious vaginal opening (64–71); antiandrogen: androgen-receptor binding and altered androgen-dependent outcomes (testosterone levels, anogenital distance, cryptorchidism, hypospadias, retention of nipples) (71–75); progesterone: reduced progesterone levels in rabbits (25), reduced progesterone synthesis in granulosa cells in vitro (76, 77), inhibited progesterone-induced reporter gene activity in vitro (78)	Persistent vaginal estrus in mice and rats (23, 68, 79, 80)	Decreased ovulation rate in rabbits (25), absence of corpora lutea (23), increase in follicular cysts (24), and ovarian hypertrophy in rats (22)
Lindane	Antiandrogen: lowered testosterone secretion (81–91); progesterone: reduced progesterone levels in ewes (26) and mice (92); FSH/LH: disturbances in LH and LH pulse frequency (26, 27, 82, 93) in ewes, rams, and rats as well as an increase in FSH in rats (27)	Disturbance of the estrous cycle in ewes and rats (26, 87, 94–97)	Reduced number of corpora lutea in ewes (26), reduced ovulation rates in rabbits (25), and disrupted ovarian cyclicity in rats (27)
Mancozeb/ maneb	Thyroid: influence on thyroid hormone levels and thyroid weight in rats (28, 98, 99)	Reduced number of estrous cycles and increased duration of diestrus in rats (28, 29)	Decreased number of healthy follicles and number of corpora lutea in the rat ovary (28, 29)

* Six of 50 pesticides examined in the Agricultural Health Study showed evidence of probable hormonal activity in vivo or in vitro or effects on the ovary or estrous cycle in vivo.

† FSH, follicle-stimulating hormone; LH, luteinizing hormone; DDT, dichlorodiphenyltrichloroethane.

‡ DDT, dichlorodiphenyldichloroethylene (DDE), (dichlorodiphenyl) acetic acid (DDA), dichlorodiphenyldichloroethane (DDD), and 2,2-bis(*p*-chlorophenyl)ethanol (DDOH).

carbaryl (16–21), dichlorodiphenyltrichloroethane (DDT) (22–25), lindane (25–27), and mancozeb (28, 29) affect the ovary by reducing the number of corpora lutea, disrupting ovarian cyclicity, and increasing the number of follicular cysts. These and other pesticides (carbon tetrachloride (30–34) and maneb (35, 36)) act as reproductive hormone agonists or antagonists in both in vitro and in vivo studies. We examined data from the Agricultural Health Study, a longitudinal study of farmers and their wives residing in Iowa and North Carolina, to determine whether exposure to these specific hormonally active and ovotoxic pesticides was associated with timing of menopause among women between the ages of 35 and 55 years living and working on farms.

MATERIALS AND METHODS

Data for this study were derived from the Agricultural Health Study (37) sponsored by the National Cancer Institute, the National Institute of Environmental Health Sciences, and the US Environmental Protection Agency (www.aghealth.org). The Institutional Review Board at the National Institutes of Health approved the study. Over 50,000 farmers and commercial pesticide applicators were recruited through pesticide licensing agencies in North Carolina and Iowa between 1993 and 1997. After enrollment, married farmers brought two questionnaires home for their spouses to complete: the Spouse Questionnaire, which inquired about pesticide exposures, demographic characteristics, and general health; and the Female and Family Health Questionnaire, which inquired about reproductive health. Approximately 32,000 farmers' spouses participated in the study by completing the Spouse Questionnaire. Female licensed applicators completed an Enrollment Questionnaire, an Applicator Questionnaire, and the Female and Family Health Questionnaire. Figure 1 details the questionnaires completed and the study population included in the analysis.

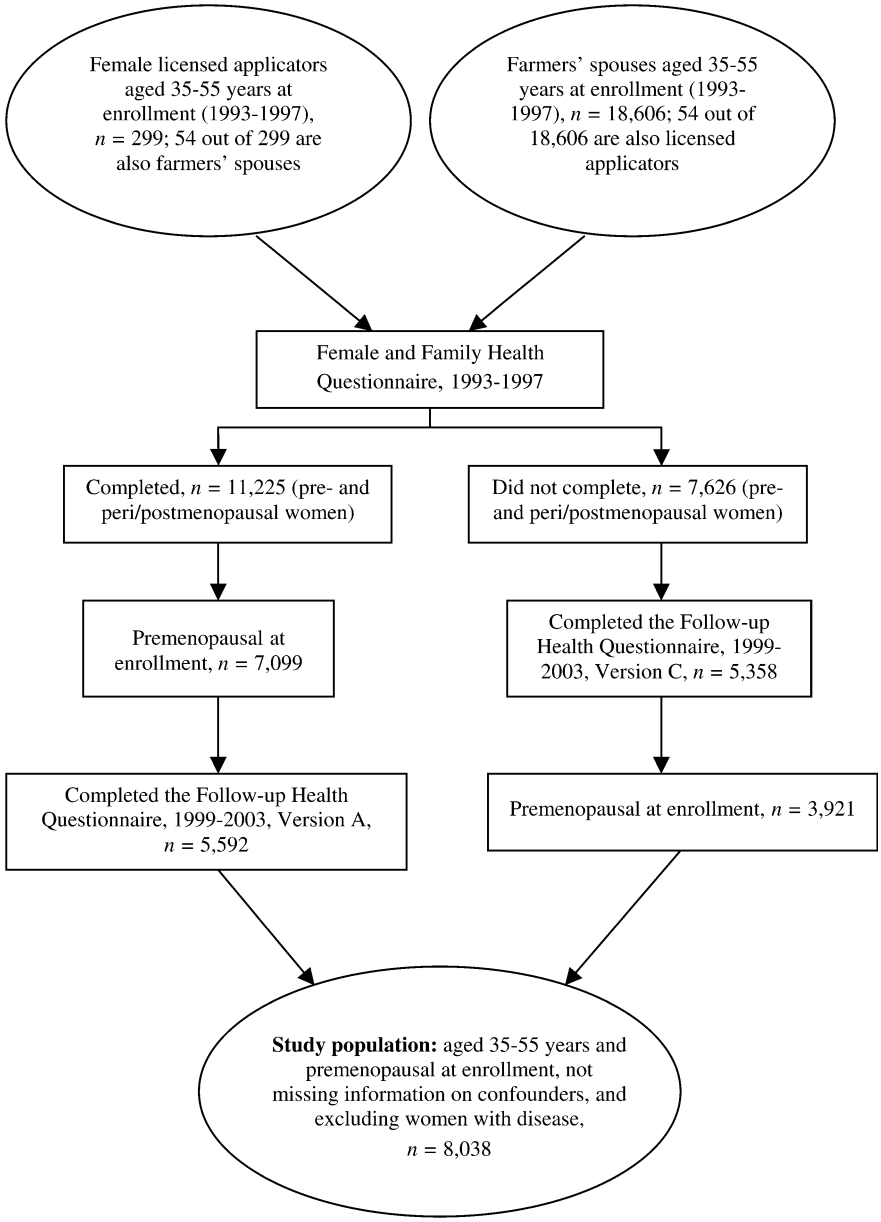

FIGURE 1. Design of the Agricultural Health Study, Iowa and North Carolina, 1993–2003. At enrollment, 54 women were both female farmers and spouses of farmers.

Follow-up interviews in the Agricultural Health Study began in 1999 and concluded in 2003. Women answered questions in the Follow-up Health Questionnaire about their current reproductive health. Data for the current study were derived from women aged 35–55 years at enrollment who completed both the Spouse or Enrollment Questionnaire and the Female and Family Health Questionnaire at enrollment (P2REL0312 and P2REL0312.01). We also included data from women who did not complete the baseline Female and Family Health Questionnaire but did complete the Follow-up Health Questionnaire (Phase 2 Prerelease 0305) and, on the basis of this information, were determined to have been premenopausal and aged 35–55 years at the baseline assessment.

Assessment of menopausal status and timing of menopause

At enrollment in the Agricultural Health Study, women were asked about their menopausal status with the question, ''Have you gone through menopause (the change of life) or had surgery that caused you to completely stop having menstrual periods?''; possible answers were ''yes,'' ''no,'' and ''unsure.'' Women who answered ''no'' were considered

premenopausal. Women were also asked whether they had ever taken hormones or estrogen for symptoms related to menopause and, if so, their age at treatment initiation.

Women who had not experienced menopause at enrollment (premenopausal women) were again asked about their menopausal status, age at last menstrual period, and, if applicable, type of menopause, at the follow-up interview. Women were also asked whether they had ''taken Premarin, estrogen, or another hormone replacement therapy like Provera'' since enrollment in the study. Women who enrolled in the study by completing the Spouse or Enrollment Questionnaires but did not complete the Female and Family Health Questionnaire at enrollment were asked additional reproductive health questions at follow-up and were included in the analyses if their responses indicated they were premenopausal at enrollment.

Exposure assessment

At enrollment, women were asked about ever personally mixing or applying 50 different pesticides. We determined what pesticides may affect reproductive hormones, the ovary, or the estrous cycle by reviewing the toxicology literature pertaining to each of the pesticides (table 1). We focused this literature review on studies showing evidence of disruption of reproductive hormones (estrogen, androgens, thyroid hormone, progesterone, follicle-stimulating hormone, and luteinizing hormone) or effects on the ovary or estrous cycle (''ovotoxic''). In the analyses, we classified women who, at enrollment, answered ''yes'' to ever mixing or applying any hormonally active or ovotoxic pesticide listed in table 1 as exposed. We also examined any pesticide exposure (ever/never), pesticides classified by functional group (herbicide, crop insecticide, livestock insecticide, fungicide, or fumigant) or chemical class (anilide, carbamate, dinitroaniline, organochlorine, organophosphate, phenoxy herbicide, and triazine), and average number of days per year mixing and applying pesticides during the time period that the woman worked on the farm for women who had ever used hormonally active or ovotoxic pesticides (0 days, 1–9 days, $\geq$10 days). In all analyses, women who had never mixed or applied pesticides at enrollment were considered unexposed regardless of their exposure status at follow-up. We also repeated the analyses by excluding women who had initiated pesticide use between enrollment and follow-up; however, results did not change, and these women were considered ''unexposed'' in all analyses.

Study population

A total of 18,851 women in the Agricultural Health Study were between the ages of 35 and 55 years at enrollment (figure 1). By December 2003, 9,513 premenopausal women had completed the follow-up interview, which is approximately 86 percent of the 11,020 premenopausal women aged 35–55 years at enrollment. At follow-up, women were again asked about their menopausal status. Women who were unsure of their menopausal status ($n = 27$) and women whose values for date or type of menopause at follow-up were missing or implausible ($n = 181$) were excluded. From self-reported data gathered at enrollment, we excluded 33 women diagnosed with diabetes before age 20 years, 351 diagnosed with thyroid or Graves' disease before age 40 years, 349 whose information on thyroid or Graves' disease was missing, 52 with scleroderma or lupus or for whom this information was missing, 117 with a previous cancer diagnosis (other than skin cancer), 23 whose information on cancer was missing, 198 whose values for smoking status ($n = 176$) or use of oral contraceptives ($n = 22$) were missing or erroneous, and 144 for whom data on pesticide use were missing or implausible. A total of 8,038 women were included in the final data set, or 73 percent of the 11,020 premenopausal women between the ages of 35 and 55 years at enrollment.

Analysis

Cox proportional hazards modeling was used to examine survival of ovarian function (time to menopause), with age at last menstrual period as the outcome variable. The proportional hazards regression procedure in SAS software (SAS Institute, Inc., Cary, North Carolina) was used to generate hazard ratios and 95 percent confidence intervals for all exposures of interest.

Premenopausal women (aged 35–55 years at enrollment) were followed from birth to their age in days at last menstrual period if postmenopausal or their follow-up interview if still premenopausal. These data were left truncated since women entered the study at different ages. To account for left truncation, women were considered at risk for the outcome only after their age at enrollment in the study. Each woman's age in days at last menstrual period was imputed in the following three ways from her self-reported age in years at last menstrual period: 1) using the woman's month and day of birth, 2) adding 6 months to the woman's month and day of birth, and 3) adding 12 months to the woman's month and day of birth. All three methods produced similar hazard ratios; therefore, we used the midyear method for all analyses. Women who experienced a surgical menopause were censored at the imputed age at surgery (last menstrual period). Women who did not experience menopause by their follow-up interview were censored at their age at the interview.

We did not have information on age at initiation of hormone replacement therapy for women who initiated therapy between enrollment and follow-up interviews ($n = 1,879$; 23 percent). Women who experienced menopause between their baseline and follow-up interviews and who used hormone replacement therapy during that time were followed to the date of their last menstrual period ($n = 1,223$; 15 percent). In separate analyses, we included and excluded women who remained premenopausal at follow-up and who used hormone replacement therapy between baseline and follow-up. There was little change in the hazard ratio for any pesticide exposure after excluding these women; therefore, data for these women were retained in the final analyses, and the women were censored at their age at the follow-up interview.

Exposures that accelerate ovarian atresia, increase the rate of ovulation, or disrupt the hormonal feedback signals affecting follicular development and ovulation may shorten the time to menopause. In contrast, exposures such as parity and longer menstrual cycles, and use of oral contraceptives, may lead to fewer ovulatory cycles and a later age at menopause (7–9). Other exposures, such as body mass index, alcohol consumption, and level of education may also be associated with timing of menopause, but mechanisms remain unclear. Using the epidemiologic literature on timing of menopause, we examined age, smoking status around the time of menopause, parity, body mass index, age at menarche, past use of oral contraceptives, education, alcohol consumption, leisure-time exercise, and thyroid disease diagnosis after age 40 years as potential confounders of a pesticide use–timing of menopause association (38, 39). We also examined whether having a license to apply restricted-use pesticides was a confounder. Since we were interested in pesticide use as the main exposure and wanted to increase precision of our final estimates, only those variables that changed the hazard ratio for any pesticide exposure by at least 10 percent, or had strong independent effects on the timing of menopause, were included in the final models. The final model controlled for smoking status (yes/no) and use of oral contraceptives (ever/never) assessed at enrollment. The observed associations with these risk factors were as follows: hazard ratio = 1.88 (95 percent confidence interval (CI): 1.59, 2.22) for smoking and hazard ratio = 0.89 (95 percent CI: 0.79, 1.02) for ever use of oral contraceptives.

We also examined proportional hazards assumptions by using Kaplan-Meier curves and interactions with time for each variable in the model. All of the variables met the conditions for proportional hazards assumptions.

RESULTS

The mean age of women at enrollment was 42 years (table 2). Almost all (99 percent) of the women in the sample were White, and the majority (56 percent) had more than a high school education. Eighty percent resided in Iowa and 20 percent in North Carolina. The women in this sample had lived or worked on a farm for an average of 28 years, 62 percent had ever mixed or applied pesticides, and 116 (2 percent) of the women had a license to apply pesticides. Twenty-six percent of women in the study experienced menopause between baseline and follow-up.

Ever mixing or applying any pesticide was associated with a later age at menopause (hazard ratio (HR) = 0.87, 95 percent CI: 0.78, 0.97) (table 3). This hazard ratio translates into an increase of approximately 3 months (84 days) in the median time to menopause for a woman who had ever mixed or applied pesticides compared with a woman who had never mixed or applied pesticides. Use of crop insecticides was associated with a later age at menopause (HR = 0.87, 95 percent CI: 0.74, 1.01), as were other pesticide classes and functional groups, although the estimates were imprecise. Results did not change when analyses were stratified by state (Iowa or North Carolina; data not shown).

Limiting the exposed group to those women who had ever mixed or applied hormonally active or ovotoxic pesticides did not change the overall result (HR = 0.86, 95 percent CI: 0.77, 0.97). However, limiting exposure to only hormonally active pesticides (excluding 480 women (75 percent) from the exposed group who had ever used only carbaryl, a pesticide with no known effects on reproductive hormones) reduced the hazard of becoming menopausal over follow-up (HR = 0.77, 95 percent CI: 0.65, 0.92), an increase of approximately 5 months (149 days) in the median time to menopause for women who had ever mixed or applied hormonally active pesticides compared with those who had never mixed or applied pesticides. Carbon tetrachloride may affect progesterone and thyroid hormones secondarily because of primary hepatic effects. Therefore, in one analysis, we excluded women who mixed or applied carbon tetrachloride and no other hormonally active pesticides ($n = 14$). This exclusion had no effect on the hazard ratio for hormonally active pesticides (data not shown). All estimates for individual hormonally active or ovotoxic pesticides were in the same range as that for use of any hormonally active or ovotoxic pesticide (HRs = 0.63–0.89). However, because of the small number of women using individual pesticides, the precision of these estimates was low.

In the analysis, 868 (29 percent) women who reported never mixing or applying pesticides at enrollment had mixed or applied pesticides by follow-up. Excluding these women from the unexposed group had a minimal effect on estimated associations: for any pesticide use, hazard ratio = 0.84 (95 percent CI: 0.74, 0.94) and, for use of hormonally active pesticides, hazard ratio = 0.74 (95 percent CI: 0.62, 0.89).

To tease out physical activity and overall health from the use of pesticides, in separate analyses we compared women who mixed or applied probable hormonally active pesticides with women who applied pesticides with no evidence of hormonal properties or limited information on their hormonal or ovotoxic properties; although slightly attenuated, results remained (HR = 0.88, 95 percent CI: 0.73, 1.07). We also analyzed the data by reproductive life span (age at menopause minus age at menarche), censoring women who remained premenopausal at follow-up, but results for ever use of pesticides remained the same (HR = 0.84, 95 percent CI: 0.75,0.93).

We also examined the same associations in cross-sectional data from 8,507 women between the ages of 40 and 60 years at enrollment in the Agricultural Health Study. Results were in the same direction as those presented here, but were less strong (HRs = 0.79–0.96) (data not shown).

DISCUSSION

To our knowledge, this study is the first to examine exposure to hormonally active or ovotoxic pesticides and timing of menopause. Atrazine, carbaryl, carbon tetrachloride, DDT, lindane, mancozeb, and maneb affect the ovary, disrupt estrous cycles, or affect reproductive hormones in animals (table 1). Ovotoxic effects of these pesticides in animals include ovarian cysts, changes in ovarian weight, and decreases in ovulation and number of corpora lutea. The sample size

TABLE 2. Demographic characteristics, pesticide use, and menopausal status of 8,038 women aged 35–55 years in the Agricultural Health Study, Iowa and North Carolina, 1993–1997

Characteristic*	Never used pesticides ($n = 3,025$)			Ever used pesticides ($n = 5,013$)		
	Mean (SD†)	No.	%	Mean (SD)	No.	%
Demographics						
Age (years)	41.9 (4.9)			42.6 (5.0)		
Pregnancies (no.)	2.9 (1.5)			3.0 (1.6)		
Missing		16	0.5		19	0.4
Race						
White		2,952	97.6		4,985	99.4
Other/multiple		70	2.3		22	0.4
Missing		3	0.1		6	0.1
Education						
<High school		59	2.0		42	0.8
High school/general equivalency diploma		1,015	33.6		1,424	28.4
>High school		1,680	55.5		2,848	56.8
Missing/other		271	9.0		699	13.9
Body mass index (weight (kg)/height (m)^2)	25.8 (5.3)			25.9 (4.9)		
Missing		1,023	33.8		1,405	28.0
Smoking status						
Never		2,214	73.2		3,681	73.4
Former		494	16.3		906	18.1
Current		302	10.0		416	8.3
Missing‡		15	0.5		10	0.2
Ever used birth control pills						
Yes		2,548	84.2		4,339	86.6
No		477	15.8		674	13.5
Age at menarche (years)						
<12		415	13.7		679	13.5
12		893	29.5		1,466	29.2
13		914	30.2		1,578	31.5
14		466	15.4		729	14.5
≥15		290	9.6		508	10.1
Missing		47	1.6		53	1.1
Alcohol consumption						
Never		1,209	40.0		1,608	32.1
<1 drink per month		902	29.8		1,637	32.7
1–3 drinks per month		525	17.4		995	19.9
1 drink per week		225	7.4		404	8.1
2–4 drinks per week		126	4.2		298	5.9
>4 drinks per week		33	1.1		56	1.1
Missing		5	0.2		15	0.3

Table continues

was large (total $n = 8,038$, and 2,124 (26 percent) experiencing a natural menopause during follow-up), allowing us to address small effects with good precision. Additionally, the women in our study are thought to be among those most highly exposed to pesticides in the United States.

Few other studies have examined the association between specific pesticide exposure and timing of natural menopause (40, 41). Cooper et al. (41) reported an increased hazard ratio (earlier age at menopause) for women with higher plasma levels of *p*,*p*′-1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (an isomer of DDE), a breakdown product of the pesticide DDT, using data from 1,407 women in a breast cancer case-control study. Akkina et al. (40) also reported associations between high serum levels of specific organochlorine

TABLE 2. Continued

Characteristic*	Never used pesticides ($n = 3,025$)			Ever used pesticides ($n = 5,013$)		
	Mean (SD)	No.	%	Mean (SD)	No.	%
Leisure-time physical activity§						
None		456	15.1		696	13.9
1–2 hours		829	27.4		1,452	29.0
3–5 hours		562	18.6		1,071	21.4
≥6 hours		311	10.3		633	12.6
Missing		867	28.7		1,161	23.2
Thyroid disease						
Yes		35	1.2		63	1.3
No		2,990	98.8		4,950	98.7
State of residence						
Iowa		2,280	75.4		4,113	82.1
North Carolina		745	24.6		900	18.0
Years lived/worked on farm (no.)	24.7 (14.2)			29.5 (13.0)		
Missing		37	1.2		154	3.1
Pesticide use						
Ever mixed or applied probable hormonally active or ovotoxic pesticides¶						
Yes		0			2,989	59.6
No		3,025	100		2,006	40.0
Missing		0			18	0.4
Days per year mixed/applied pesticides (no.)	N/A†			9.6 (17.0)		
Missing		N/A			1,104	22.0
Applicator status						
Spouse		3,020	99.8		4,902	97.8
Licensed applicator		4	0.1		89	1.8
Spouse and licensed applicator		1	0.0		22	0.4
Outcome characteristics						
Menopausal status at follow-up						
Premenopausal		2,244	74.2		3,670	73.2
Postmenopausal		781	25.8		1,343	26.8
Type of menopause						
Natural		241	30.9		375	27.9
Surgical		540	69.1		968	72.1
Age at natural menopause (years)	50.3 (3.5)			50.9 (3.5)		
Ever used hormone replacement therapy#						
Yes		413	52.9		810	60.3
No		363	46.5		528	39.3
Missing		5	0.6		5	0.4

* All characteristics were assessed at study enrollment, unless otherwise stated.
† SD, standard deviation; N/A, not applicable.
‡ These women answered "no" to the question, "Do you currently smoke cigarettes?"
§ Estimated no. of hours per week of strenuous leisure-time physical activity during the summer.
¶ Atrazine, carbaryl, carbon tetrachloride, dichlorodiphenyltrichloroethane (DDT), lindane, mancozeb/maneb.
# Among postmenopausal women at follow-up.

pesticides (*p,p′*-DDT, *p,p′*-DDE, beta-hexachlorocyclohexane, and *trans*-nonachlor) and earlier age at menopause using data from 219 women in the 1982–1984 Hispanic Health and Nutrition Examination Survey. In contrast to these studies, our analysis found that use of DDT was associated with a later age at menopause. This difference may be due to

TABLE 3. Hazard ratios and 95% confidence intervals for pesticide exposure* and timing of menopause among 8,038 women aged 35–55 years at enrollment in the Agricultural Health Study, Iowa and North Carolina, 1993–1997

Exposure	Exposed: no. experiencing menopause/total†	HR‡,§	95% CI‡
Any pesticide	968/5,013	0.87	0.78, 0.97
Functional group			
Herbicides	723/3,725	0.88	0.74, 1.05
Crop insecticides	778/3,828	0.87	0.74, 1.01
Livestock insecticides	198/1,076	0.92	0.54, 1.59
Fungicides	160/762	0.85	0.35, 2.05
Fumigants	108/531	1.44	0.64, 3.22
Pesticide class¶			
Anilide	120/538	0.63	0.36, 1.11
Carbamate	601/2,791	0.89	0.76, 1.04
Dinitroaniline	136/584	0.90	0.57, 1.40
Organochlorine	140/505	0.69	0.36, 1.31
Organophosphate	470/2,317	0.85	0.67, 1.06
Phenoxy herbicides	280/1,379	0.85	0.65, 1.11
Triazine	112/509	0.63	0.36, 1.12
Average no. of days per year mixing and applying pesticides			
0	540/3,025	1.00	
1–5	239/1,225	0.88	0.76, 1.02
6–9	155/644	0.98	0.82, 1.16
≥10	158/707	0.94	0.79, 1.11
Hormonally active or ovotoxic pesticides	644/2,989	0.86	0.77, 0.97
Hormonally active pesticides	164/682	0.77	0.65, 0.92
Atrazine	91/412	0.79	0.63, 0.99
Carbaryl	590/2,736	0.89	0.79, 1.00
Carbon tetrachloride	8/28	0.63	0.31, 1.27
DDT‡	54/125	0.82	0.62, 1.09
Lindane	29/138	0.74	0.51, 1.08
Mancozeb/maneb	26/105	0.78	0.53, 1.16

* Self-reported (ever/never) mixing or applying of 50 pesticides.

† No. of women experiencing menopause/no. of women who never used pesticides = 540/3,025 for all pesticide analyses.

‡ HR, hazard ratio; CI, confidence interval; DDT, dichlorodiphenyl-trichloroethane.

§ Data were left truncated and women were considered at risk only after their age at enrollment in the study; analyses were controlled for smoking status at enrollment (nonsmoker/smoker) and for use of oral contraceptives (never/ever).

¶ Anilides: alachlor, metolachlor; carbamates: aldicarb, benomyl, carbaryl, carbofuran; dinitroanilines: pendimethalin, trifluralin; organochlorines: aldrin, chlordane, DDT, dieldrin, heptachlor, lindane, toxaphene; organophosphates: chlorpyrifos, coumaphos, diazinon, dichlorvos, fonofos, malathion, parathion, phorate, terbufos, trichlorfon; thiocarbamates: butylate, *S*-Ethyl dipropylthiocarbamate (EPTC), ziram; phenoxy herbicides: 2,4-D, 2,4,5-T, 2,4,5-TP; triazines: atrazine, cyanazine, metribuzin.

different sampling or exposure assessment strategies or too few women exposed in the current study to allow for precise effect estimation. Our analysis was based on self-reported history of mixing and applying pesticides rather than on biomarkers of exposure, and a total of 125 women in this analysis had ever used DDT.

The toxicology evidence, along with the two existing epidemiologic studies examining DDE and timing of menopause (40, 41), led us to hypothesize that use of hormonally active or ovotoxic pesticides would result in an earlier menopause in women by depleting germ cells in the ovary. However, there was no evidence in our analyses that use of these

pesticides led to an earlier menopause. On the contrary, taken together, use of hormonally active pesticides was associated with later age at menopause. Later age at menopause is associated with fertility in later reproductive years and possibly with an increased risk of certain reproductive cancers but a decreased risk of cardiovascular disease and overall mortality (3, 8). A recent study reported a reduction in age-adjusted all-cause mortality by 2 percent per year increase in age at menopause (40). In our study, use of pesticides was associated with a delay of 3–5 months in timing of menopause.

Number of ovulatory cycles has been inversely associated with age at menopause (7), while cycle length has been positively associated with age at menopause (9). Additional analyses among younger women in the Agricultural Health Study showed that exposure to hormonally active pesticides was associated with longer menstrual cycles and more missed menstrual periods (42). Thus, pesticide exposure may lead to a later age at menopause through effects on menstrual cycles. Alternatively, hormonally active pesticides may directly affect timing of menopause through effects on follicle-stimulating hormone and luteinizing hormone. In contrast to these findings, this and other studies have found that smoking is associated with shorter menstrual cycles among premenopausal women (44–47) and with a younger age at menopause (5, 48) (or, in the type of analyses presented here, an increased hazard ratio). The mechanisms through which smoking affects menstrual cycles and timing of menopause may be different from those through which other exposures affect these outcomes.

Women who use pesticides may be healthier overall than women who do not use pesticides. If a later age at menopause is associated with a woman's health status, use of our comparison could lead to a spurious association between pesticide use and timing of menopause. We excluded from the analysis women with diagnosed cancers, women who may have undergone chemotherapy, and women with autoimmune diseases that may be associated with timing of menopause. Additionally, our measure of exposure was ever/never mixing or applying specific pesticides in a woman's lifetime, which should capture in the exposed group any women who may have initially worked on the farm, became ill over time, and were no longer able to perform farm work. Our comparison group was composed of women who also live and work on farms but never mix or apply pesticides, rather than women in the general population. We also examined the association of use of probable hormonally active pesticides with use of pesticides with limited or no evidence of hormonal properties, and, although attenuated, results remained in the same direction. These factors should lessen any bias from a healthy worker effect that may be present in our analysis.

Some women ($n = 1{,}507$) who were premenopausal at baseline were excluded from the analysis because they did not complete the follow-up questionnaire and their menopausal status at that time could not be determined. These women were similar in age and past history of oral contraceptive use to women in the analysis; however, slightly more were current smokers (13 percent excluded from analysis vs. 9 percent included in analysis), and fewer had ever mixed or applied pesticides (54 percent excluded from analysis vs. 62 percent included in analysis). It is unclear how this exclusion may have affected the results.

Women stated whether they had ever mixed or applied specific pesticides in their lifetime; more detailed information on pesticide exposure was not available. Therefore, we do not know at what time in their reproductive lives, how often, or in what quantities women were exposed to the specific pesticides. We had data on average number of days personally mixing or applying pesticides but no data on the number of days that the women used specific pesticides. These limitations may have caused some exposure misclassification if women used pesticides for only a short time; however, this scenario would attenuate any effect estimates.

The results of our study suggest that use of certain pesticides may lead to a later age at menopause. Further research, using prospective designs that include more detailed data on timing and dose of exposures, measures of physical activity and other potential confounders, and more frequent assessment of menopausal status, would be a useful follow-up to these findings.

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