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AGE AT NATURAL MENOPAUSE AND EXPOSURE TO ORGANOCHLORINE PESTICIDES IN HISPANIC WOMEN

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A cross-sectional study was conducted to evaluate the relationship between exposure to selected organochlorine pesticides (OCP) (p,p′-*DDT,* p,p′-*DDE, dieldrin, hexachlorobenzene, beta-hexachlorocyclohexane [beta-HCH], oxychlordane,* trans-*nonachlor) and age at natural menopause in a sample of 219 menopausal women participating in the Hispanic Health and Nutrition Examination Survey in 1982–1984. Information on age at menopause, reproductive history, demographic variables, and potential confounding variables was collected via interview. Analysis of variance was employed to compare adjusted mean age at natural menopause among women by category of serum OCP level. Serum levels of* p,p′-*DDT,* p,p′-*DDE, beta-HCH, and* trans-*nonachlor were associated with a younger age at menopause. In particular, women with exposure levels in the highest exposure categories (serum* p,p′-*DDT* $\geq$ *6 ppb, beta-HCH* $\geq$ *4 ppb, or* trans-*nonachlor* $\geq$ *2 ppb) had an adjusted mean age at menopause on average 5.7, 3.4, and 5.2 yr earlier, respectively, than women with serum levels of these pesticides below the detection limit. Women with serum* p,p′-*DDE levels greater than 23.6 ppb (highest quintile) had an adjusted mean age at menopause 1.7 yr earlier than women with serum* p,p′-*DDE levels less than 5.5 ppb (lowest quintile). However, no consistent dose-response effect was apparent across low, medium, and high exposure categories. Interactions were detected for* p,p′-*DDT in combination with beta-HCH,* trans-*nonachlor, or oxychlordane, as well as beta-HCH in combination with oxychlordane.*

Many organochlorine pesticides (OCPs), such as dichlorodiphenyltrichlorethane (DDT), and their metabolites have been identified as endocrine disruptors with the potential to affect reproductive processes in humans and animals (NRC, 1999). Organochlorine pesticides are persistent environmental contaminants with multiple routes of human exposure, including inhalation, oral, and dermal; however, dietary exposure and gastrointestinal absorption are considered the most important routes (NRC, 1999). The lipophilicity and long half-life of these compounds lead to their distribution and sequestration in the

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body according to the lipid content of tissues, with levels in adipose tissue 200–400 times those in serum (Needham et al., 1990). The use of many OCPs has been banned in the United States and other developed countries, but they continue to be found in environmental media (NRC, 1999).

Endocrine-disrupting mechanisms associated with organochlorine pesticides include (1) steroid hormone receptor binding, (2) altered hormone transport and clearance, and (3) altered hormone postreceptor activation, including second-messenger systems, which are important for the action of many peptide hormones (Crisp et al., 1998). For example, DDT has been shown to bind the estrogen receptor, and the DDT metabolite dichlorodiphenyldichloroethylene (DDE) has been shown to bind both the estrogen and androgen receptor (Danzo, 1997; Cooper & Kavlock, 1997). Dieldrin and chlordane are reported to disrupt cell membrane Ca^{2+} transport mechanisms involved in some second-messenger systems (Mehotra et al., 1989; ATSDR, 1992b). Beta-hexachlorocyclohexane activates tyrosine kinase, another second messenger (Hatakeyama & Matsumura, 1999).

Age at natural menopause is an important reproductive parameter and has been associated with morbidity and mortality risk (Snowdon et al., 1989). Women experiencing an early menopause are at increased risk for cardiovascular disease, stroke, and osteoporosis, and at decreased risk for breast cancer (Snowdon et al., 1989; Kelsey et al., 1993). Natural menopause is defined as the permanent cessation of menstruation resulting from the loss of ovarian follicular activity (Sowers & La Pietra, 1995). There are two perspectives on the biologic processes leading to menopause. One perspective focuses on the exhaustion of the pool of growing ovarian follicles as the initiating event, which then results in hypothalamic–pituitary changes. The second gives more importance to age-related changes in the central nervous system in the initiation of menopause, with exhaustion of ovarian follicles resulting from these neuroendocrine changes (Wise et al., 1996).

Many demographic, lifestyle, and reproductive factors have been associated with age at menopause (Table 1). The magnitude of the effect of most of these factors on early menopause is in the range of 1 to 2 years. Most can be explained by their effect on ovarian follicle depletion or damage.

The effects of exposure to pesticides on age at menopause have not been extensively examined. However, living in a rural setting (compared to an urban setting) has been significantly associated with a shorter reproductive lifespan in women, due to an earlier age at natural menopause (Garry et al., 2002). Recently, Cooper et al. (2002) reported that women with higher plasma *p,p′*-DDE levels had a median age of onset of natural menopause earlier by about 1 year. Further, OCPs have been found in ovarian follicular fluid at levels similar to serum concentrations (Baukloh et al., 1985; Jarrel et al., 1992). It was hypothesized that the endocrine-disrupting characteristics of OCPs may alter the hormonal milieu in the ovaries or the neuroendocrine system, potentially affecting oocyte depletion and therefore the timing of menopause. This cross-sectional epidemiologic study analyzed data from the Hispanic Health and

TABLE 1. Factors Associated With Age at Natural Menopause

Factor	Reference
Factors associated with early menopause	
African-American race	Torgerson et al. (1994)
Earlier birth year	Do et al. (1998)
Maternal early menopause	Torgerson et al. (1994)
Menstrual cycles <26 d	Sowers and La Pietra (1995), Bromberger et al. (1997)
Irregular menstrual cycles	Bromberger et al. (1997)
Late age at menarche (>14 yr)	Do et al. (1998)
Nulliparity	Do et al. (1998), Torgerson et al. (1994)
Never using oral contraceptives	Gold et al. (2001)
Lower social class/education	Gold et al. (2001), Torgerson et al. (1994)
Nonemployment	Gold et al. (2001)
Separated, widowed, divorced	Gold et al. (2001)
Smoking	Do et al. (1998); Gold et al. (2001); Bromberger et al. (1997); Sowers and La Pietra (1995); Torgerson et al. (1994); McKinlay et al. (1992)
Never using alcohol	Torgerson et al. (1994)
Weight reduction dieting	Bromberger et al. (1997)
Lower meat consumption	Torgerson et al. (1994)
Autoimmune disorders	Vermuelen (1993)
History of heart disease	Gold et al. (2001)
Irradiation	Vermuelen (1993)
Chemotherapy	Greendale and Sowers (1997)
Residence at high altitude	Vermuelen (1993), Flint (1997)
Residence in south or north central United States	Stanford et al. (1987)
Factors associated with late menopause	
Japanese race	Gold et al. (2001)
Parity	Vermuelen (1993); Gold et al. (2001)
Oral contraceptive use	Van Noord et al. (1997); Gold et al. (2001)
Higher social class/income/education	Do et al. (1998)
Higher intake of yellow and green vegetables	Nagata et al. (2000)

Nutrition Examination Survey (HHANES) to evaluate the association between age at menopause and exposure to specific organochlorine pesticides or their metabolites.

SUBJECTS AND METHODS

Population

The HHANES was conducted by the National Center for Health Statistics in 1982–1984 (U.S. DHHS, 1985). The HHANES was a survey of three Hispanic subgroups of the U.S. population residing in selected areas of the United States (U.S. DHHS, 1985; Delgado et al., 1990). The three Hispanic subgroups and the geographic areas where they were sampled were: Mexican Americans residing in Arizona, California, Colorado, New Mexico, and Texas; Cuban Americans residing in Dade County, Florida; and Puerto Ricans residing in the New York City metropolitan area including parts of New York, New Jersey, and Connecticut (U.S. DHHS, 1985). This study was based on the HHANES database because a large proportion of this population was expected to have been exposed to OCPs. The majority of the HHANES participants were Mexican American, and human monitoring studies of OCP residues in Mexico have indicated a high degree of exposure to many OCPs (Albert et al., 1980; Waliszewski et al., 1998). In addition, persons residing in the southern or western United States have been shown to have an increased likelihood of having quantifiable serum levels of many OCPs (Stehr-Green, 1989). Information on reproductive history and many important potential confounding variables was also available in the database. Information on some factors potentially associated with age at menopause, such as chemotherapy, radiation, and hormone replacement therapy, were not available. For the purposes of the present study, persons of non-Hispanic origin and non-White race were excluded since the number of non-Hispanic or non-White persons was too small for adequate analysis.

The data collection methods utilized in the HHANES included interviews, direct physical examination, anthropometry, diagnostic testing, and laboratory analyses. Interviews were administered in the household and within a mobile examination center. All examinations and testing were conducted in the mobile examination center located in the community (U.S. DHHS, 1985; Delgado et al., 1990).

In the HHANES, serum pesticide analyses were conducted for a half-sample of adults (20–74 yr of age) and a half-sample of adolescents aged 12–19 yr (U.S. DHHS, 1985). Among the 1599 Hispanic females aged 12–74 yr with serum pesticide data, 22% were 36–50 yr of age and 20% were greater than 50 yr of age. Of these 1599 women, 393 responded affirmatively to a question asking if their periods had stopped entirely, not counting during pregnancy. In addition, they were asked at what age their periods had stopped entirely, and if they had had a hysterectomy or oophorectomy. Of the 393 women reporting that their period had stopped entirely, 173 were excluded from analysis due to surgical menopause (hysterectomy, oophorectomy, or both). Additionally, there were 23 women who reported age at menopause of less than 35 yr; because these responses were considered spurious, data for these 23 women were excluded from analysis, leaving 219 naturally menopausal women for analyses.

Exposure Assessment

Exposure assessment employed in the HHANES survey is described in detail elsewhere (U.S. DHHS, 1992). Briefly, nonfasting blood samples were obtained from subjects at the examination center. All serum OCP analyses were conducted at the U.S. Environmental Protection Agency Pesticide Monitoring Laboratory in Bay St. Louis, MS. The analytical methodology involved extracting the

OCP from serum with hexane (no cleanup step was included) and analyzing by packed-column gas chromatography (GC) with an electron-capture detector.

In total, 18 OCPs and metabolites were analyzed (U.S. DHHS, 1992). The specific pesticides/metabolites, along with their limits of quantitation in parts per billion (ppb) in brackets, were: *trans*-nonachlor [1], heptachlor epoxide [1], oxychlordane [1], heptachlor [2], alpha-hexachlorocyclohexane [1], beta-hexachlorocyclohexane [1], gamma-hexachlorocyclohexane [1], delta-HCH [1], aldrin [2], endrin [2], dieldrin [1], *o,p′*-DDT [2], *p,p′*-DDT [2], *o,p′*-DDE [1], *p,p′*-DDE [1], *o,p′*-DDD [2], *p,p′*-DDD [2], and hexachlorobenzene [1]. Only 7 compounds were detected in more than 1% of the HHANES serum samples: *p,p′*-DDT, *p,p′*-DDE, dieldrin, oxychlordane, beta-hexachlorocyclohexane (beta-HCH), hexachlorobenzene (HCB), and *trans*-nonachlor. These seven organochlorine pesticides/metabolites were selected as the exposures of interest for this study because they are identified in the toxicologic literature as potential endocrine disruptors or reproductive toxicants (NRC, 1999; ATSDR, 1991, 1992a, 1992b, 1992c, 1994) and were detected in an adequate proportion of the serum samples for statistical analysis. Serum polychlorinated biphenyls (PCBs), which were measured at a detection limit of 15 ppb, were detected in less than 1% of the sample and therefore were not included in the analysis.

Demographic, Lifestyle, and Reproductive Variables

To control for confounding in the analyses, the following demographic, lifestyle, and reproductive variables potentially associated with either age at menopause or body burden of OCPs were extracted from the interview questionnaire data: age at the time of examination (blood draw), Hispanic group (Mexican American, Puerto Rican, Cuban), marital status (ever or never), education (>8th grade, ≤8th grade), family income (≥$20,000/yr, <$20,000/yr), cigarette smoking (never or ever at least 100 cigarettes in entire life), alcohol consumption (ever or never), born in the United States (yes or no), current residence in central city area of a Standard Metropolitan Statistical Area (SMSA) (yes or no), farm work (ever or never), parity (0, 1–5, 6–10, 11–15), oral contraceptive use (ever or never), age at menarche, and age at natural menopause. Alcohol consumption data were obtained from the food frequency questionnaire. Two physiologic variables potentially associated with body burden of OCPs were also evaluated: serum cholesterol level and body mass index (BMI; weight in kilograms divided by the square of height in meters). Previous work has shown that it is important to adjust for serum lipids in studies of OCPs (Philips et al., 1989). Serum cholesterol was the only available marker of serum lipids in the database. Therefore, where appropriate, adjustment for serum cholesterol was included in the analysis.

Statistical Analysis

To describe the demographic, lifestyle, and reproductive characteristics of the sample, frequencies and proportions were determined for all categorical variables by Hispanic subgroup. For continuous variables, such as age, serum cholesterol level, BMI, serum OCP levels, and age at menopause, the mean, standard deviation, median, and range were determined. The Shapiro–Wilk statistic and a normal probability plot were used to test for normality.

Analysis of variance (ANOVA) was employed to compare mean age at menopause by category of serum OCP level. Because the number of subjects with detectable levels and the range of levels varied substantially among OCPs, multiple categorization schemes were used. First, most OCPs were categorized into three levels: below the detection limit (BDL); detection limit up to median concentration; and above the median concentration. Second, to capture the upper end of the distribution, an integer categorization scheme using the concentration in parts per billion in whole numbers (e.g., 1.00–1.99 ppb) was used. Third, for *p,p′*-DDE there was an adequate sample size to evaluate dose response using quintiles. Except for *p,p′*-DDE, the referent group consisted of those women with BDL serum OCP levels. Because all but two women had detectable serum *p,p′*-DDE, the referent group for the *p,p′*-DDE categorization was the lowest quintile. Next, an analysis of covariance (ANCOVA) model, which included the continuous variable age at examination, was fit separately for each OCP exposure variable. Other potential confounders were also screened using ANCOVA models. Potential confounding factors identified in screening with $p \leq .25$ were then included together in a multiple-factor ANCOVA model. Variables with $p < .10$ were considered to be important potential confounders and were included in the adjusted models for each separate exposure variable. Interaction among exposure variables found to be associated with age at menopause was evaluated. The sample was not large enough to permit separate analyses for the three groups of Hispanic women; however, a variable for Hispanic group was included in the multivariate analysis to adjust for confounding. Because results were generally similar for unadjusted and adjusted analyses, only adjusted results are presented.

RESULTS

The mean age at the time of examination of the 219 menopausal women was 58.7 yr. The mean age at menopause for Mexican American, Cuban, and Puerto Rican women was 47.4 yr, 49.6 yr, and 48.6 yr, respectively. The difference in mean age at menopause between the three Hispanic groups was statistically significant ($p = .01$). The distribution of demographic, lifestyle, and reproductive variables is shown in Table 2, both overall and for each of the three Hispanic subgroups. Almost 70% of the sample had less than a 9th-grade education. Slightly more than one-third of the women had ever smoked cigarettes, and slightly more than one-third ever drank alcohol. None of the Cuban or Puerto Rican women were born in the United States. In contrast, 54.5% of Mexican American women were born in the United States. Approximately 7% of the women were nulliparous, with the majority of women having had from 1 to 5 births. Only 14% of the women reported a history of oral contraceptive use.

The descriptive statistics for the serum OCP levels are presented in Table 3. The proportion of women with serum OCP levels above the detection limit

TABLE 2. Characteristics of Menopausal Women by Hispanic Group, HHANES, 1982–1984 ($n = 219$)

Characteristic	Mexican American *n* (%)	Cuban *n* (%)	Puerto Rican *n* (%)	Total *n* (%)
Age at examination (yr)				
42–49	10 (8.0)	3 (5.8)	2 (4.8)	15 (6.9)
50–54	32 (25.6)	10 (19.2)	14 (33.3)	56 (25.6)
55–59	33 (26.4)	18 (34.6)	13 (30.9)	64 (29.2)
60–64	17 (13.6)	9 (17.3)	6 (14.3)	32 (14.6)
65–69	16 (12.8)	2 (3.9)	6 (14.3)	24 (10.9)
70–74	17 (13.6)	10 (19.2)	1 (2.4)	28 (12.8)
Age at menopause				
35–39	11 (8.8)	1 (1.9)	0	12 (5.5)
40–44	16 (12.8)	2 (3.8)	5 (11.9)	23 (10.5)
45–49	43 (34.4)	17 (32.7)	17 (40.5)	77 (35.2)
50–54	47 (37.6)	25 (48.1)	18 (42.9)	90 (41.1)
55–57	8 (6.4)	7 (13.5)	2 (4.8)	17 (7.8)
Education				
≤8th grade	89 (72.4)	27 (52.9)	29 (70.7)	145 (67.4)
>8th grade	34 (27.6)	24 (47.1)	12 (29.3)	70 (32.6)
Income				
<$20,000/yr	98 (84.5)	36 (70.6)	35 (83.3)	169 (80.9)
≥$20,000/yr	18 (15.5)	15 (29.4)	7 (16.7)	40 (19.1)
Marital status				
Ever married	120 (96.8)	48 (92.3)	41 (97.6)	9 (4.1)
Never married	4 (3.2)	4 (7.7)	1 (2.4)	209 (95.9)
Residence				
Not SMSA-central city	56 (44.8)	30 (57.7)	6 (14.3)	92 (42.0)
SMSA-central city	69 (55.2)	22 (42.3)	36 (85.7)	127 (58.0)
Country of birth				
Not United States	56 (45.5)	52 (100)	42 (100)	150 (69.1)
United States	67 (54.5)	0	0	67 (30.9)
Occupation				
Ever farm work	35 (28.0)	3 (5.8)	5 (11.9)	43 (19.6)
Never farm work	90 (72.0)	49 (94.2)	37 (88.1)	176 (80.4)
Smoker (more than 100 cigarettes in life)				
Ever	45 (36.0)	18 (34.6)	13 (31.0)	76 (34.7)
Never	80 (64.0)	34 (65.4)	29 (69.0)	143 (65.3)
Alcohol drinker				
Ever	39 (31.7)	19 (36.5)	5 (12.8)	63 (29.4)
Never	84 (68.3)	33 (63.5)	34 (87.2)	151 (70.6)
Parity				
0	7 (5.6)	9 (17.3)	0	16 (7.3)
1–5	60 (48.4)	42 (80.8)	29 (69.0)	131 (60.1)
6–10	43 (34.7)	1 (1.9)	12 (28.6)	56 (25.7)
11–15	14 (11.3)	0	1 (2.4)	15 (6.9)
Oral contraceptive use				
Ever	21 (16.8)	4 (7.7)	5 (11.9)	30 (13.7)
Never	104 (83.2)	48 (92.3)	37 (88.1)	189 (86.3)

TABLE 3. Distribution and Univariate Statistics for OCPs in Serum for Women by Hispanic Group, HHANES 1982–1984

OCP, detection limit (ppb)	*n*	*n* (%) Above detection limit	Minimum, maximum[a] (ppb)	Median[a], Mean[a] (ppb)
Beta-HCH 1.00				
All groups	219	87 (39.7)	1.00, 9.37	2.1, 2.39
Mexican American	125	71 (56.8)	1.00, 5.30	1.97, 2.25
Cuban	52	11 (21.2)	1.57, 9.37	2.61, 3.36
Puerto Rican	42	5 (11.9)	1.46, 3.55	1.96, 2.34
p,p′-DDE 1.00				
All groups	219	217 (99.1)	1.00, 169.31	28.60, 35.65
Mexican American	125	125 (100)	2.40, 168.00	38.45, 43.05
Cuban	52	52 (100)	4.16, 169.31	22.63, 30.96
Puerto Rican	42	40 (95.2)	2.41, 71.55	17.64, 20.58
p,p′-DDT 2.00				
All groups	219	73 (33.3)	2.00, 15.24	3.50, 4.24
Mexican American	125	57 (45.6)	2.00, 15.24	3.32, 4.15
Cuban	52	16 (30.8)	2.06, 10.68	4.06, 4.58
Puerto Rican	42	0		
Dieldrin 1.00				
All groups	219	19 (8.7)	1.00, 3.90	1.54, 1.84
Mexican American	125	18 (14.4)	1.00, 3.30	1.42, 1.72
Cuban	52	1 (1.9)	3.9	
Puerto Rican	42	0		
HCB 1.00				
All groups	219	14 (6.4)	1.09, 5.67	1.68, 2.20
Mexican American	125	11 (8.8)	1.09, 2.57	1.32, 1.57
Cuban	52	3 (5.8)	3.03, 5.67	4.81, 4.50
Puerto Rican	42	0		
Oxychlordane 1.00				
All groups	219	15 (6.9)	1.00, 3.97	1.20, 1.47
Mexican American	125	12 (9.6)	1.00, 1.87	1.20, 1.27
Cuban	52	3 (5.8)	1.00, 3.97	1.82, 2.26
Puerto Rican	42	0		
trans-Nonachlor 1.00				
All groups	219	37 (16.9)	1.09, 3.25	1.56, 1.69
Mexican American	125	28 (22.4)	1.09, 3.25	1.61, 1.75
Cuban	52	8 (15.4)	1.16, 2.10	1.39, 1.53
Puerto Rican	42	1 (2.4)	1.4	

[a] Includes only values above detection limit.

ranged from 99.1% for serum *p,p′*-DDE to 6.4% for hexachlorobenzene. The serum concentrations for *p,p′*-DDE ranged from 1 ppb to 169.31 ppb. For the other 6 OCPs, the means for above detection limits (ADL) OCP concentrations were much lower, ranging from 1.47 ppb to 4.24 ppb. For all seven OCPs examined, the proportion of women above the detection limit was highest among Mexican Americans and lowest among Puerto Ricans. The mean serum concentrations of beta-HCH, hexachlorobenzene, and oxychlordane, however, were higher among Cuban compared to Mexican American women (Table 3).

A significant decrease in mean age at menopause was found for the highest exposure category in the adjusted analysis by median split for *p,p′*-DDT, by integer categorization for *trans*-nonachlor, and by both median split and integer categorization for beta-HCH (Table 4). Serum levels of dieldrin, hexachlorobenzene, and oxychlordane were not associated with age at menopause when adjusted for potentially important covariates (age at examination, Hispanic group, education, and age at menarche). Women with serum *p,p′*-DDT level ≥ 6 ppb had an adjusted mean age at menopause 5.65 yr earlier than women with serum *p,p′*-DDT BDL. Women with serum beta-HCH ≥ 4 ppb had an adjusted mean age at menopause 3.35 yr earlier than women with serum beta-HCH BDL. Women with serum *trans*-nonachlor ≥ 2 ppb had an adjusted mean age at menopause 5.18 yr earlier than women with serum *trans*-nonachlor BDL. An association of borderline significance ($p = .13$) was found between high serum *p,p′*-DDE level and earlier menopause in the adjusted model. Women with serum *p,p′*-DDE level ≥ 23.60 ppb (highest quintile) had an adjusted mean age at menopause 1.68 yr earlier than women with a level less than 5.46 ppb (lowest quintile).

When interaction terms which represented the combined effect of two OCPs were added to the adjusted ANOVA models, a number of significant interactions were identified (Table 5). Women with *p,p′*-DDT ADL plus either beta-HCH, oxychlordane, or *trans*-nonachlor ADL had a mean age at menopause approximately 3–5 yr lower than those who had neither OCP detected in their serum, and 2–4 yr lower than women who had only DDT in their serum. Women with both serum beta-HCH ADL and oxychlordane ADL had a mean age at menopause approximately 4 yr earlier than those with neither OCP.

DISCUSSION

The average age at natural menopause in the United States and in European populations ranges from 48 to 52 years (Sowers & La Pietra, 1995). For this sample of Hispanic women, the mean age at menopause of 48.1 yr is at the low end of the range for the general U.S. population. Garrido-Latorre et al. (1996) reported a low mean age of menopause of 46.5 yr in a sample of Hispanic women residing in Mexico City. A recent study of factors associated with age at menopause, however, did not find an early age at menopause for Hispanic women residing in the United States (Gold et al., 2001). The low mean age at menopause found in this study may be explained by the low socioeconomic status, low educational attainment, and developing country nativity of the majority of the sample population, since all are risk factors for early menopause (Gold et al., 2001; Gonzales et al., 1997). The median serum levels of the seven OCPs observed in the menopausal women in this study are similar to median levels observed in adult male and female participants in the

TABLE 4. Mean[a] Age at Menopause by Each Organochlorine Pesticide Exposure Variable, HHANES 1982–1984

Exposure	ppb	*n*	Mean ± SE	*p* Value
Beta-HCH				
Median split				
Below detection limit	<1.00	128	48.45 ± 0.43	ref
Above detection limit to median	1.00–2.09	41	48.67 ± 0.76	.80
Above median	>2.09	43	46.83 ± 0.74	.07
Integer categorization	<1.00	128	48.47 ± 0.42	ref
	1.00–1.99	39	48.43 ± 0.78	.97
	2.00–2.99	27	46.54 ± 0.90	.06
	3.00–3.99	10	50.11 ± 1.50	.28
	≥4.00	8	45.12 ± 1.68	.06
p,p′-DDT				
Median split				
Below detection limit	<2.00	142	48.80 ± 0.40	ref
Above detection limit to median	2.00–3.43	34	47.76 ± 0.82	.27
Above median	>3.43	36	46.04 ± 0.79	<.01
Integer categorization	<2.00	142	48.81 ± 0.40	ref
	2.00–2.99	28	47.67 ± 0.87	.24
	3.00–3.99	16	48.54 ± 1.14	.82
	4.00–5.99	13	46.70 ± 1.29	.12
	≥6.00	13	43.16 ± 1.27	<.01
Dieldrin				
Median split				
Below detection limit	<1.00	193	48.24 ± 0.34	ref
Above detection limit to median	1.00–1.30	9	48.28 ± 1.59	.98
Above median	> 1.30	10	48.61 ± 1.52	.30
Integer categorization	<1.00	193	48.24 ± 0.34	ref
	1.00–1.99	12	47.32 ± 1.39	.52
	≥2.00	7	47.56 ± 1.80	.71
HCB				
Median split				
Below detection limit	<1.00	198	48.24 ± 0.33	ref
Above detection limit to median	1.00–1.32	6	46.35 ± 1.96	.34
Above median	>1.33	8	47.71 ± 1.66	.75
Integer categorization	<1.00	198	48.24 ± 0.33	ref
	1.00–1.99	9	46.96 ± 1.63	.45
	≥2.00	5	47.44 ± 2.11	.71
Oxychlordane				
Median split				
Below detection limit	<1.00	199	48.28 ± 0.33	ref
Above detection limit to median	1.00–1.13	4	44.86 ± 2.33	.15
Above median	>1.13	9	47.08 ± 1.57	.45
trans-Nonachlor				
Median split				
Below detection limit	<1.00	177	48.34 ± 0.35	ref
Above detection limit to median	1.00–1.50	18	48.08 ± 1.10	.82
Above median	>1.50	17	46.42 ± 1.15	.11

TABLE 4. Mean[a] Age at Menopause by Each Organochlorine Pesticide Exposure Variable, HHANES 1982–1984 (*Continued*)

Exposure	ppb	*n*	Mean ± SE	*p* Value
Integer categorization	<1.00	177	48.34 ± 0.35	ref
	1.00–1.99	27	48.48 ± 0.89	.88
	≥2.00	8	43.16 ± 1.64	<.01
p,p′-DDE quintile				
	<5.46	41	48.19 ± 0.77	ref
	5.46–10.43	43	48.78 ± 0.70	.57
	10.44–16.09	43	49.35 ± 0.71	.27
	16.10–23.60	42	47.99 ± 0.72	.85
	>23.60	43	46.51 ± 0.73	.13

Note. BDL, below detection limit; ADL, above detection limit; ref, referent category.
[a] Least-squares mean adjusted for age at examination, Hispanic group, education, and age at menarche.

TABLE 5. Mean[a] Age at Menopause by Binary Combinations[b] of Organochlorine Pesticide Exposure Variables, HHANES 1982–1984

Organochlorine pesticides		*n*	Mean ± SE	*p* Value
p,p′-DDT	beta-HCH			
0	0	100	48.42 ± 0.48	ref
1	0	28	48.93 ± 0.85	.60
0	1	42	49.78 ± 0.70	.12
1	1	42	45.44 ± 0.75	<.01
p,p′-DDT	Oxychlordane			
0	0	138	48.70 ± 0.40	ref
0	1	4	52.13 ± 2.27	.14
1	0	61	47.36 ± 0.61	.08
1	1	9	43.62 ± 1.52	<.01
p,p′-DDT	*trans*-Nonachlor			
0	0	131	48.69 ± 0.41	ref
0	1	11	50.43 ± 1.40	.23
1	0	46	47.45 ± 0.69	.13
1	1	24	45.65 ± 0.97	<.01
beta-HCH	Oxychlordane			
0	0	125	48.38 ± 0.42	ref
0	1	3	52.87 ± 2.66	.09
1	0	74	48.13 ± 0.56	.73
1	1	10	44.31 ± 1.49	.01

Note. 0, Below detection limit; 1, above detection limit; ref, referent category.
[a] Least-squares means adjusted for age at examination, Hispanic group, education, and age at menarche.
[b] Those combinations for which the two-way interaction term was statistically significant ($p \leq 0.05$).

NHANES II (National Health Examination and Nutrition Study), conducted in the United States in 1976–1980 (Murphy & Harvey, 1985). The proportion with detectable levels of beta-HCH, HCB, oxychlordane, or *trans*-nonachlor, however, was higher in the HHANES sample of menopausal women, compared to the NHANES II sample (Murphy & Harvey, 1985).

In this study, serum levels of *p,p′*-DDT, *p,p′*-DDE, beta-HCH, and *trans*-nonachlor were associated with an earlier age at menopause. The decrease in age at menopause for these individual OCPs was clearly evident between the BDL category compared to the highest exposure category; however, there was no consistent dose-response relationship apparent in the middle categories. This could be due to either the small sample size or a threshold effect. Interactive effects were associated with binary combinations of *p,p′*-DDT and either beta-HCH, *trans*-nonachlor, or oxychlordane, as well as the binary combination of beta-HCH and oxychlordane. The association found in this study between higher serum *p,p′*-DDE levels and a younger age at menopause is similar to that reported by Cooper et al. (2002).

One biologically plausible explanation for the effect of *p,p′*-DDT, beta-HCH, and *trans*-nonachlor on age at menopause is ovarian damage potentially produced by endocrine disruption or adverse effects due to chronic lifetime exposure to these OCPs, which are stored in adipose tissue and are found in ovarian follicular fluid at levels similar to serum concentrations (Baukloh et al., 1985; Jarrel et al., 1992). In utero exposure is unlikely, because these women who had experienced menopause by 1982–84 were born prior to 1940 when these OCPs began to be manufactured (ATSDR, 1991, 1992a, 1992b, 1992c, 1994). Natural menopause is thought to be due to the depletion of ovarian follicles responsive to the gonadotropins (Sowers & La Pietra, 1995). Damage to the ovarian follicles or oocytes or interference with their normal development may lead to an early menopause (Sowers & La Pietra, 1995). The hypothalamus and pituitary gland and changes in the pattern of their neuroendocrine messages may also play an important role in the timing of menopause (Wise et al., 1996).

Evidence from *in vitro* studies suggests potential ovarian effects for *p,p′*-DDT. *In vitro* studies have demonstrated a depressive effect of *p,p′*-DDT and gamma-HCH on oocyte maturation and an effect of decreased bovine oviductal cell viability for *p,p′*-DDT (Alm et al., 1998; Tiemann et al., 1998). DDT and chlordane are reported to affect ion transport across cell membranes, including inhibition of calcium transport which may affect the action of peptide hormones (ATSDR, 1992a, 1992b). Dieldrin, DDT, chlordane, and gamma-HCH are reported to inhibit gap-junction intercellular communication (Zhong-Xiang et al., 1986; Aylsworth et al., 1989; ATSDR, 1992b), which may affect growth and maturation of oocytes (Goodenough et al., 1999; Li & Mather, 1997; Anderson & Albertini, 1976; Gilula et al., 1979).

Evidence that OCPs may affect central endocrine targets is provided in a study of OCPs and repeated miscarriages by Gerhard et al. (1998). These authors reported correlations between OCPs with markers of thyroid and pituitary function. They found inverse associations between thyroid-stimulating hormone and beta-HCH, gamma-HCH, PCBs, and *p,p′*-DDE, and positive correlations of follicle-stimulating hormone and prolactin with total OCP body burden.

Other potential endocrine-disrupting mechanisms of action of OCPs may involve estrogenic or antiestrogenic activity, as well as changes in metabolism and circulating levels of steroid hormones (Crisp et al., 1998). Although *p,p′*-DDT can bind to cellular estrogen receptors and is considered a weak estrogen (Danzo, 1997), beta-HCH does not bind to the estrogen receptor but has been found to exert estrogenic effects through an influence on the estrogen receptor (Steinmetz et al., 1996). Chlordane can affect metabolism and circulating levels of steroid hormones (ATSDR, 1992b).

There are limitations of this study that necessitate viewing the study findings cautiously. A limitation inherent in a cross-sectional study is that the temporal relationship between the exposure and outcome cannot be established. An alternative explanation for the association between serum OCPs and early menopause is that changes in fat metabolism and serum lipids resulting from menopause itself may produce an increase in body burden or circulating levels of serum OCPs (Roberts & Silbergeld, 1995). Another possibility is that an underlying metabolic abnormality in some women could independently result in both elevated OCP levels and early age at menopause.

Another limitation is that the OCP exposures measured at the time of sampling may not represent the level of exposure at the time of menopause. The time interval between age at menopause and age at examination in this study ranged from 0 to 37 yr, with a mean of 10.6 yr and a median of 8.0 yr, indicating that the distribution was positively skewed. OCPs have long half-lives (e.g., the median half-life of beta-HCH in serum is 7.2 yr) (Jung et al., 1997) and biological measures of OCPs reflect long-term exposure; however, changes in exposure, metabolism, or excretion over time may have led to misclassification of exposure at the time of menopause. Another possible source of exposure misclassification may be related to lactation, a major route of OCP excretion. A lactation history was not available in the HHANES data. All women in the analysis were menopausal at the time of exposure measurement; therefore, the time interval between menopause and the exposure measurement did not include pregnancy or lactation. However, the data did not allow us to distinguish between women with low levels of OCPs throughout their life and women who may have had high OCP levels during childhood and adolescence and then reduced those levels—for example, through lactation—during adulthood.

Potential confounders, such as type of oral contraceptive, hormone replacement therapy, and tobacco and alcohol consumption, may not have been adequately accounted for in the analysis due to the manner in which the data were collected and thus may have resulted in residual confounding. Further, the data for many variables, such as age at menopause, were collected retrospectively and may have been misclassified due to dependence on the participant's memory. However, misclassification from this source is likely to be nondifferential with respect to the exposures of interest (serum OCPs), since each participant was unaware of her OCP level. Data were stratified by age at examination (>56 yr vs. ≤ 56 yr), and no significant difference was found in results across strata, suggesting that systematic recall bias was probably not present.

In this study of women who had experienced natural menopause, high serum levels of *p,p′*-DDT, *p,p′*-DDE, beta-HCH, and *trans*-nonachlor were found to be associated with a younger age at menopause. The relationship between age at menopause and exposure to OCPs deserves further study.

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