American Journal of Epidemiology
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Vol. 164, No. 11
DOI: 10.1093/aje/kwj329
Advance Access publication October 5, 2006

Original Contribution

A Prospective Study of Serum DDT and Progesterone and Estrogen Levels across the Menstrual Cycle in Nulliparous Women of Reproductive Age

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Received for publication December 7, 2005; accepted for publication May 1, 2006.

The authors explored whether exposure to 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) and its isomers and metabolites affects female reproductive hormones characterized by urinary pregnanediol-3-glucuronide (PdG) and estrone conjugate (E_1C) levels. During 1996–1998, 287 newly married Chinese women nonsmokers intending to conceive were prospectively studied. Serum for DDT measurement was collected at enrollment, and daily menstrual diaries and urine specimens were collected for 1 year or until a clinical pregnancy was achieved. More than 500 menstrual cycles were studied totaling over 8,000 days. Day of ovulation was determined for each cycle, and the association of serum DDT levels with daily PdG and E_1C levels in a $\pm$10-day window around ovulation was analyzed. After adjustment for covariates including age, body mass index, and occupational exposures, consistent inverse associations of most DDT forms occurred with urine E_1C during the periovulation phase and with urine PdG during the luteal phase of the menstrual cycle. For example, a 10-ng/g increase in serum *p,p′*-DDE was associated with a 0.05-log(E_1C) decrease ($p = 0.03$) in the periovulation phase and a 0.06-log(PdG) decrease ($p = 0.03$) in the luteal phase. These results support the potential for DDT to be associated with decrements in estrogen and progesterone levels at times during the menstrual cycle that are critical for ovulation and early pregnancy maintenance.

DDT; dichlorodiphenyl dichloroethylene; endocrine; estrogens; estrone; hormones; pregnanediol-3alpha-glucuronide; progesterone

Abbreviations: DDD, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane; DDE, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene; DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; E_1C, estrone conjugates; PdG, pregnanediol-3-glucuronide.

Exogenous substances or mixtures that alter the structure or function of the endocrine system are referred to as endocrine-disrupting chemicals (1). Because of their chemical stability, lipophilic nature, and propensity to bioaccumulate, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) and its various forms remain ubiquitous contaminants in food, human adipose tissue, and human breast milk (2), and DDT is still used in some countries for malaria control (3). DDT

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compounds enter the circulatory system, are transported via the lipid component in plasma, and can be measured in serum (4).

Because 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE) and 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (DDD) are degradation and metabolic products of DDT, humans are usually exposed to a mixture of these three compounds (5). In addition, DDT, DDE, and DDD can each exist in different isomeric forms determined by the chlorine position on the two chlorophenyl rings of the molecule (5). Technical-grade DDT typically consists of 77 percent *p*,*p*′-DDT, 15 percent *o*,*p*′-DDT, 4 percent *p*,*p*′-DDE, and less than 1 percent *o*,*p*′-DDE, *p*,*p*′-DDD, and *o*,*p*′-DDD (6).

Results of in vitro and in vivo rodent studies suggest that the *o*,*p*′ isomers can act as estrogen agonists, whereas the *p*,*p*′ isomers have androgen antagonist activity (5). However, in competitive binding assays, *o*,*p*′-DDT and its metabolites *o*,*p*′-DDD, *o*,*p*′ DDE, as well as *p*,*p*′-DDT, were able to bind to the human estrogen receptor in yeast MCF-7 cells (7). In other experimental models, *p*,*p*′-DDT and *p*,*p*′-DDE had higher affinities for the human progesterone receptor than for the human estrogen receptor (alpha), indicating that interaction with the progesterone receptor may contribute to the endocrine-disrupting properties associated with certain DDT forms (8).

Few epidemiologic studies have evaluated the effects of total DDT, DDE, or DDD exposure on female reproductive function. Windham et al. (9) recently reported a luteal phase length decrease of 0.6 days for each doubling of *p*,*p*′-DDE level and a significant decrease in progesterone metabolite levels with increasing *p*,*p*′-DDE quartile levels among 48 Southeast Asian immigrant women of reproductive age living in San Francisco, California.

We investigated the association of serum total DDT, DDE, and DDD concentrations with levels of urinary pregnanediol-3-glucuronide (PdG; the major metabolite of progesterone) and estrone conjugates (E_1C; the major metabolites of estrogen) in a cohort of women participating in a study of environmental organochlorine exposure and reproductive health in Anhui, China. Our prior investigations in this cohort showed that the relative odds of early pregnancy losses associated with a 10-ng/g increase in serum total DDT was 1.17 (95 percent confidence interval: 1.05, 1.29) (10), and, compared with the lowest quartile, the odds of experiencing a shortened menstrual cycle among women in the highest serum total DDT quartile was 2.85 (95 percent confidence interval: 1.12, 7.31) (11). To extend this work, we examined whether DDT, DDE, or DDD was associated with female reproductive hormones critical for fertility and pregnancy maintenance, specifically progesterone and estrogen.

MATERIALS AND METHODS

Study population and procedures

Study subjects were a subset of women participating in a prospective cohort study of reproductive health and rotating-shift work among female textile workers in Anhui, China. All women were newly married, were aged 20–34 years, were nulliparous, and had obtained state permission to have a child (12). A nonfasting serum sample was collected at baseline prior to when the women had stopped contraception with the intent to conceive. Beginning from the date of stopping contraception and continuing for up to 12 months or until pregnancy was clinically confirmed, whichever occurred first, each woman kept a daily diary to record menses and sexual intercourse and collected a first-morning urine specimen for hormone metabolite measurements.

For the parent study, of 1,006 newly married women who were screened (more than 90 percent of newly married women employed at the mill), 971 met the enrollment criteria, and 961 were enrolled and had baseline data. We excluded 496 enrolled women from this analysis because of incomplete data. The characteristics of the excluded women were similar to those of women who were included (12). Of the remaining 465 women, 387 provided adequate daily urine and diaries and had urinary measures of PdG and E_1C. Conception was assessed in all cycles by measuring urinary human chorionic gonadotropin. However, when selecting cycles for PdG and E_1C analyses, we preferentially chose cycles during which clinical pregnancy or early pregnancy loss occurred and the cycles immediately preceding them, regardless of outcome. We also included a sample of nonconceptive cycles with the fewest missing urine samples. For these 387 women with complete urinary hormone measures, archived baseline serum samples for 301 (555 cycles) were available for DDT measurement. Forty-five cycles (representing 14 women) were excluded because the day of ovulation could not be reliably estimated, leaving 510 cycles (from 287 women) for analysis.

The day of ovulation was identified to allow us to control for the expected variability in reproductive hormone levels across normal menstrual cycles. We used two methods to determine the day of ovulation. We first used the ''estrogen/progesterone algorithm,'' which identified 5-day sequences in which the ratio value (of urinary E_1C to PdG levels) for the first day was the highest of the 5 and the ratio values for each of the last 2 days were 40 percent or less of the first-day value. The second day in the sequence was designated the day of ovulation (13). We also used the ''PdG-rise algorithm,'' which used a piecewise regression model for daily PdG levels and applied a ''best fit'' (maximum R^2) criterion to identify the turning point when PdG started to rise; we assigned this as the day of ovulation (14). The estrogen/progesterone algorithm sometimes identified multiple 5-day blocks. When this occurred, we used the estimated day of ovulation closest to that identified by the PdG-rise algorithm. We conducted our analyses twice by using the day of ovulation estimated by each algorithm and did not see differences in the direction or magnitude of the results. Results using the PdG-rise method are presented here.

Laboratory assays of DDT isomers and metabolites

Nonfasting blood samples were collected when each study participant enrolled, and the serum fraction was removed and frozen at −20°C until extraction. Analyses were conducted at the Harvard School of Public Health analytic chemistry laboratory. Details of the laboratory methods

and quality control procedures are reported elsewhere (15). Serum samples were analyzed for *p*,*p*′ and *o*,*p*′ isomers of DDT, DDE, and DDD. Primary analyses of serum extracts used gas chromatography with electron capture detection, with confirmatory analyses of all samples using a capillary column of different polarity. Quantification was based on the response factor of each analyte relative to an internal standard. Final levels were reported as the mean of the two measures. However, if the two measures differed by more than 20 percent or a coeluting compound was present, the lower value or the one without interference by a coeluting compound was reported. Results were reported in units of nanograms of analyte per gram of serum after subtracting the amount of analyte measured in the procedural blank.

The lipid content of each serum sample was not measured because the sample volume was insufficient (0.5 ml). Analyses were performed in batches of approximately 18 serum samples, and each batch included a procedural blank, matrix spike samples, and laboratory control sample to assess interbatch variability. Matrix spike samples were used to calculate analyte recoveries, with mean values for most DDT compounds ranging from 95 percent to 101 percent. Because only about 50 percent of *p*,*p*′-DDD eluted into the analytic solution after column chromatography, *p*,*p*′-DDD values were adjusted for percent recovery values. The within-batch coefficient of variation for *p*,*p*′-DDE was 5 percent (15), and the mean relative percent difference for the matrix spike duplicates was 7 percent or lower for all DDT forms. The method detection limits were below 0.04 ng/g for all DDT forms. The analyst was blinded to the hormone status of the samples.

Laboratory assays of urinary PdG, E_1C, and human chorionic gonadotropin

Urinary PdG and E_1C were measured by enzyme-based immunoassays (16). The minimum detection levels for PdG and E_1C were 3 ng/ml and 0.096 ng/ml, respectively, and the coefficients of variation measured from the repeated standards were 4.3 percent and 5.1 percent, respectively. Urinary human chorionic gonadotropin levels were analyzed by the immunoradiometric assay (17). Urine creatinine levels were measured according to the Jaffe reaction (18). All PdG, E_1C, and human chorionic gonadotropin values were normalized to creatinine values to adjust for urine concentration. All urine specimens from each woman were assayed in duplicate in a single run. Discrepancies of more than threefold between duplicate assays were presumed to result from technical error, and the assay was repeated. Final concentrations were reported as the geometric mean of the duplicate analyses and were expressed in units of nanogram per milligram of creatinine.

Statistical analysis

Analyses were first conducted by using total DDT, calculated as the sum of *p*,*p*′-DDE, *o*,*p*′-DDE, *p*,*p*′-DDT, *o*,*p*′-DDT, and *p*,*p*′-DDD. We created two groups according to whether a woman's serum total DDT level was below (low group) or equal to or above (high group) the median population level. We compared characteristics of the two groups by using *t* tests for continuous variables and chi-square tests for categorical variables. We focused on a 20-day window starting from 9 days before ovulation to 10 days after ovulation for comparison of hormone profiles in the two DDT groups. To assess whether hormone profiles changed in response to conception, we first stratified our analysis by the conceptive status of the cycle. We calculated daily mean levels of urinary PdG and E_1C for the low and high total DDT groups by using the LSMEAN function in the SAS procedure GENMOD (SAS Institute, Inc., Cary, North Carolina) with 19 indicator variables in the model giving day-specific means for each day in the 20-day window and plotted these values. The distribution of urinary PdG and E_1C were strongly skewed toward the upper end, so log-transformed values were used for subsequent analyses.

We created smooth plots of serum total DDT and both log(PdG) and log(E_1C) by using penalized splines in generalized additive mixed models (19) to adjust for correlations among urine hormone metabolite levels in multiple samples from the same woman and with adjustment for day relative to ovulation.

Because both in vitro and in vivo rodent studies have found different reproductive and developmental effects depending on the DDT form, we modeled the linear associations of total DDT and each individual isomer and metabolite separately with log(E_1C) and log(PdG) by using generalized estimating equations to accommodate correlations in hormone concentrations of multiple urine samples from the same woman (20) and with adjustment for day relative to ovulation. We estimated model parameters both with and without adjustment for age (linear and quadratic terms), body mass index (linear and quadratic terms), education (high school/middle school), and answers to the following questions: among the people who live with you, do they smoke cigarettes around you on most days? (yes/no), does your job involve working different shifts? (yes/no), how stressed do you feel at your workplace? (low, moderate, high stress), how much noise are you exposed to at your workplace? (low, moderate, high levels), and how much dust are you exposed to at your workplace? (low, moderate, high levels). DDT exposure variables were also modeled as quadratic terms but were not significant and were kept as continuous terms in the multivariate linear models.

To explore whether we could identify DDT isomers or metabolites having the strongest impact on urinary reproductive hormone levels, we assessed individual effect estimates for each DDT isomer and metabolite while simultaneously adjusting for all other DDT forms, the 20-day window, and the above-specified covariates. To determine whether hormone levels were influenced by 50 cycles from 28 women who had experienced early pregnancy loss (determined by using human chorionic gonadotropin), we stratified by early pregnancy loss in conception cycles, and we investigated the association of serum total DDT with PdG and E_1C adjusting for the 20-day window and the above-specified covariates.

TABLE 1. Characteristics of 287 nulliparous textile workers who did not smoke, by serum total DDT*,†, Anhui, China, 1996–1998

	Low total DDT ($n = 143$)	High total DDT ($n = 144$)	Two-sided p value‡
Total DDT (median (range))	21.0 (5.5–29.6)	42.5 (29.6–113)	
	Mean (standard deviation)		
Total DDT (ng/g)	19.6 (6.4)	46.4 (15.2)	<0.01
Age (years)	24.7 (1.4)	25.2 (1.8)	<0.01
Height (cm)	157.4 (5.2)	157.7 (5.2)	0.61
Weight (kg)	49.5 (5.8)	49.0 (6.1)	0.44
Body mass index (kg/m^2)	20.0 (2.1)	19.7 (2.0)	0.19
	No. (%)§	No. (%)§	
Education			0.04
Middle school or below	98 (68.5)	81 (56.3)	
High school or above	45 (31.5)	63 (43.8)	
Passive smoke exposure			0.85
No	58 (40.6)	61 (42.4)	
Yes	85 (59.4)	83 (57.6)	
Noise exposure level			0.54
Low	33 (23.1)	41 (28.7)	
Moderate	56 (39.2)	50 (35)	
High	54 (37.8)	52 (36.4)	
Dust exposure level			0.13
Low	43 (30.1)	48 (33.6)	
Moderate	62 (43.4)	46 (32.2)	
High	38 (26.6)	49 (34.3)	
Perceived stress level			0.28
Low	99 (69.2)	90 (62.5)	
Moderate or high	44 (30.8)	54 (37.5)	
Shift work			0.51
No	3 (2.1)	6 (4.2)	
Yes	140 (97.9)	138 (95.8)	

* DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane.

† Total DDT = *o*,*p*′-DDT + *o*,*p*′-1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE) + *p*,*p*′-DDT + *p*,*p*′-DDE + *p*,*p*′-1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (DDD). Total DDT levels below the median (29.61 ng/g) were classified as low; levels at or above the median were classified as high.

‡ *t* test for continuous variables and chi-square test for categorical variables.

§ Some percentages do not total 100 because of rounding.

RESULTS

Study population and exposure

This analysis included 287 women who contributed data on a total of 510 cycles and over 8,000 menstrual days. The average number of cycles followed was 1.8 (range, 1–7). We compared demographic, occupational, and exposure characteristics in this sample with those of the 14 women excluded from the analyses because of greater than 3 days' discordance in ovulation day identified by our two methods; we found no significant differences (data not shown).

Table 1 describes characteristics of the sample stratified by low and high total DDT (ng/g) using a median cutpoint. Women in the high total DDT group were older ($p < 0.01$) and had a higher educational level ($p < 0.05$) than the low total DDT group.

All samples had DDT levels above the method detection limits. Table 2 shows the distributions of total DDT (ng/g) and other DDT forms. Serum total DDT levels were high compared with those reported in contemporaneous Western populations (3). The predominant form was *p*,*p*′-DDE, followed by *p*,*p*′-DDT. Correlation coefficients among the

TABLE 2. Distributions of preconception serum levels (ng/g) of serum total DDT*, isomers, and metabolites in 287 nulliparous textile workers who did not smoke, Anhui, China, 1996–1998

	Minimum	Quartile 1	Median	Quartile 3	Maximum
Total DDT	5.52	20.96	29.61	42.63	113.30
p,p′-DDT	0.37	0.99	1.47	2.23	13.12
o,p′-DDT	0.04	0.12	0.17	0.24	1.49
p,p′-DDE	4.76	19.30	27.71	39.58	97.54
o,p′-DDE	0.03	0.06	0.09	0.12	1.07
p,p′-DDD	0.07	0.15	0.20	0.29	0.96

* Total 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) = *o,p′*-DDT + *o,p′*-1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE) + *p,p′*-DDT + *p,p′*-DDE + *p,p′*-1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (DDD).

different DDT forms ranged from 0.28 (*o,p′*-DDE with *p,p′*-DDD) to more than 0.99 for total DDT with *p,p′*-DDE.

Relation between serum total DDT, PdG, and E_1C

Of the 510 cycles included in this study, 255 were nonconceptive and 255 were conceptive. Figure 1 illustrates daily mean E_1C and PdG levels over the 20-day window of the menstrual cycle, stratified by conception status using the PdG rising point to determine day of ovulation. For both conceptive and nonconceptive cycles, women with high serum total DDT levels had lower PdG and E_1C levels than women with low serum total DDT. Although these plots suggested a potentially stronger association in conceptive cycles, tests for interaction of total DDT with conception status for both E_1C and PdG were not significant. Therefore, in subsequent analyses, we combined conceptive and nonconceptive cycles. After adjustment for cycle day and covariates, there was a consistent overall inverse relation of serum total DDT with urine PdG and E_1C levels for all cycles (figure 2).

PdG-specific findings

There was a significant inverse association between total DDT and log(PdG) levels with a 10-ng/g increase in DDT associated with a 0.06-log(PdG) decrease in the periovulation phase ($p = 0.04$) and a 0.05-log(PdG) decrease in the luteal phase ($p = 0.03$) of the menstrual cycle (table 3). *p,p′*-DDE followed the same pattern of associations as total DDT; among the other DDT forms, higher serum *p,p′*-DDD was consistently associated with lower log(PdG) levels across all phases of the menstrual cycle. Except for *o,p′*-DDT, all other DDT forms were significantly associated with log(PdG) declines in the luteal phase (table 3).

When we examined log(PdG) levels in relation to all DDT forms in the same model (data not shown), adjusting for the same covariates as in table 3, *o,p′*-DDE was the only form that showed a significant inverse association with log(PdG) during all phases.

E_1C-specific findings

A 10-ng/g increase in total DDT was associated with a 0.04-log(E_1C) decrease in the periovulation ($p = 0.02$) and luteal ($p = 0.04$) phases, and *p,p′*-DDE had the same pattern of associations (table 3). All DDT forms were inversely and significantly associated with log(E_1C) in the periovulation phase. In addition, a 10-ng/g increase in *o,p′*-DDE was associated with a 8.15-log(E_1C) decrease in the luteal phase ($p = 0.02$).

When we examined log(E_1C) in relation to all DDT forms in the same model (data not shown), adjusting for the same covariates as in table 3, none of the forms was significantly associated with log(E_1C) levels.

Analyses accounting for conception and early pregnancy loss

Results were the same when the above linear regression analyses of total DDT versus log(PdG) and log(E_1C) were repeated, adjusted for conception status. Similar results were found after excluding early pregnancy loss cycles and in analyses restricted to conceptive cycles with or without exclusion of early pregnancy loss cycles. There was no association of serum total DDT with log(PdG) or log(E_1C) in analyses restricted to early pregnancy loss cycles, but we had limited power in this analysis because of a small sample size ($n = 50$ early loss cycles; data not shown).

DISCUSSION

We found significant inverse associations between serum total DDT (and individual DDT isomers and metabolites) and subsequent urine levels of PdG and E_1C in this sample of 287 reproductive-age women. Overall, the most consistent DDT-associated declines in urinary PdG and E_1C occurred during the menstrual cycle phase when these hormone metabolite levels were peaking (figure 1, table 3). For example, a significant inverse association of almost all DDT forms with urine PdG occurred during the luteal phase and with urine E_1C during the periovulation phase (table 3). The observed associations appeared to be independent of conception status.

Fluctuations in hormone levels observed during normal menstrual cycles reflect carefully timed feedback loops among gonadotropins and sex hormones that are critical for successful ovulation and corpus luteal function for early pregnancy maintenance. Our findings suggest the potential for DDT to be associated with decrements in estrogen levels at a time when rising estrogen levels are required for ovulation. Our findings also suggest the potential for DDT to be associated with lower progesterone levels at a time when rising progesterone levels are an important indicator of corpus luteal function necessary for early pregnancy maintenance. These results suggest the potential for the observed DDT-PdG and E_1C associations to have downstream consequences on female reproductive health including, for example, risk of impaired fertility and early pregnancy loss. Our previous work in this study population demonstrated

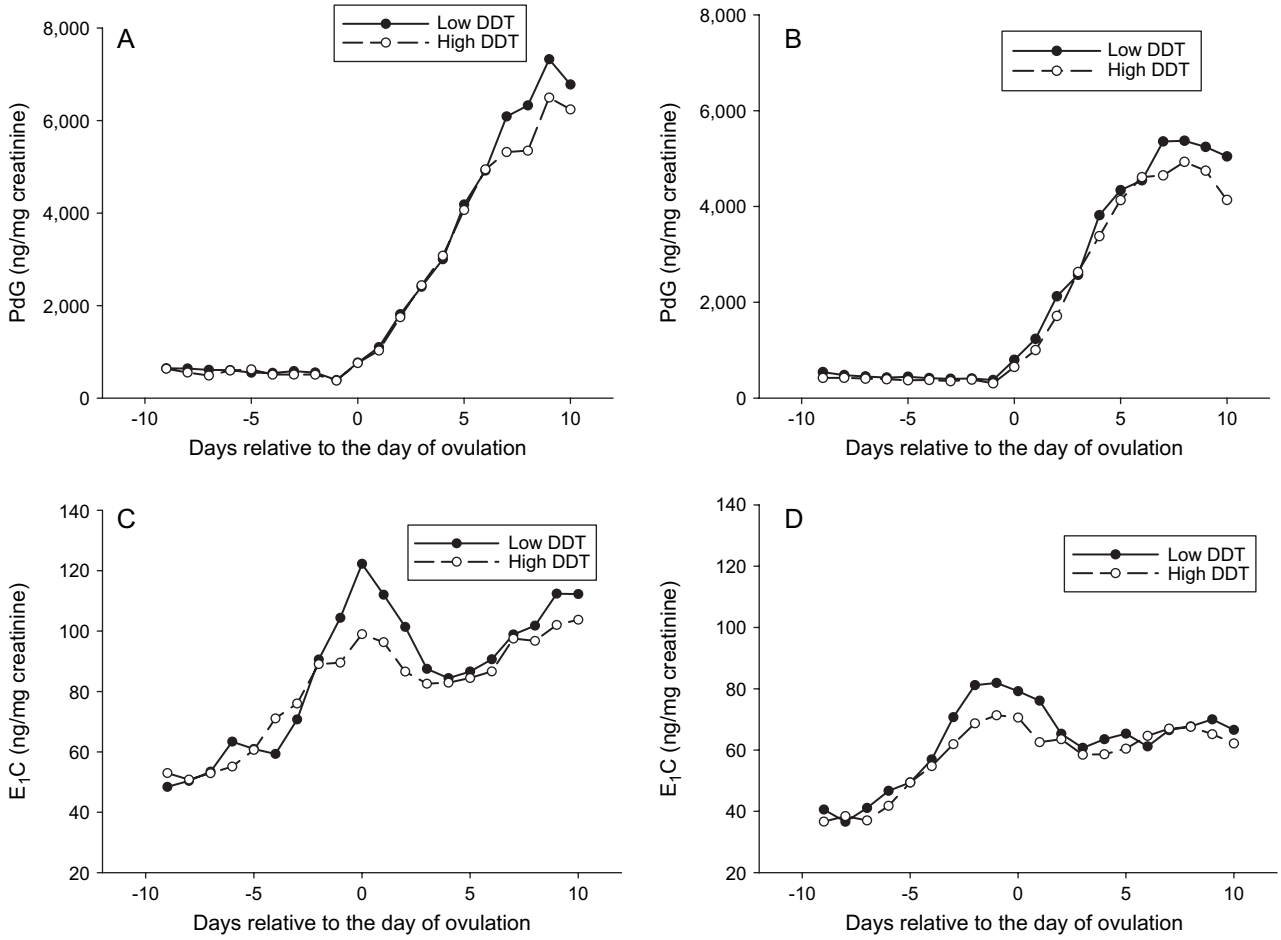

FIGURE 1. Unadjusted daily mean urinary pregnanediol-3-glucuronide (PdG) and estrone conjugate (E_1C) levels in the 20-day window around ovulation (day 0) stratified by low or high serum total 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) in 287 female textile workers who did not smoke, Anhui, China, 1996–1998. A and C, conception cycles: low-DDT group $n = 117$ cycles; high-DDT group $n = 138$ cycles. B and D, nonconception cycles: low-DDT group $n = 123$ cycles; high-DDT group $n = 132$ cycles. Total DDT = *o*,*p*′-DDT + *o*,*p*′-1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE) + *p*,*p*′-DDT + *p*,*p*′-DDE + *p*,*p*′-1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (DDD). Total DDT levels below the median (29.61 ng/g) were classified as low; levels at or above the median were classified as high.

associations of DDT with risk of pregnancy loss (10) and shortened menstrual cycle length (11).

Because of potential mechanistic differences among DDT forms, we assessed total DDT as well as individual DDT isomers and metabolites in these analyses. Our results support potential differential sensitivity of urinary PdG and E_1C levels to different DDT forms. For example, the DDT–urinary PdG relation appeared to be most consistently demonstrable for *p*,*p*′-DDE, *o*,*p*′-DDE, and *p*,*p*′-DDD, whereas the DDT–urinary E_1C relation appeared to be most consistently demonstrable for *p*,*p*-DDE and *o*,*p*′-DDE (table 3). However, because of colinearity among DDT forms, it is difficult to assess their possible independent effects with certainty.

We observed these associations at serum DDT levels substantially higher than those likely in contemporaneous US populations. The median serum *p*,*p*′-DDE level in our study was equivalent to 5,542 ng/g of lipid (estimated, assuming 0.5 percent lipid in serum) compared with 270 ng/g of lipid reported in 1,027 US women aged 12 years or older assessed in the National Health and Nutrition Examination Survey between 1999 and 2000 (21). Across the range of DDT levels observed in this cohort (minimum total DDT level of 5.5 ng/g of serum), however, there did not appear to be an effect threshold (figure 2).

The DDT-PdG association we observed is consistent with findings from one of the only other epidemiologic studies to evaluate associations of DDT with female reproductive hormones. Windham et al. (9) studied 48 Southeast Asian immigrant women living in San Francisco and found associations of serum *p*,*p*′-DDE, and to a lesser extent *p*,*p*′-DDT, with lower urinary progesterone metabolite levels

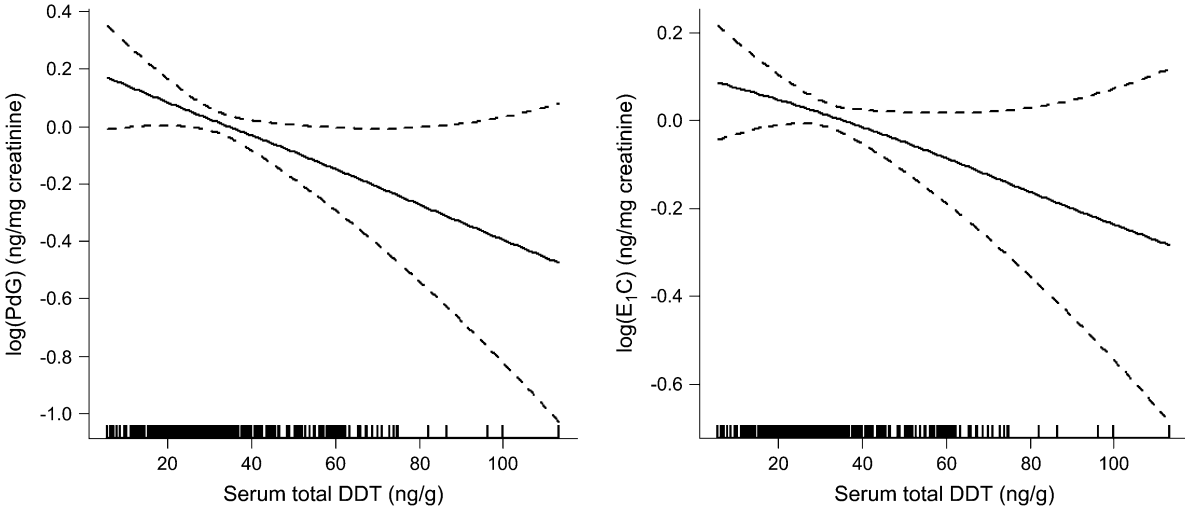

FIGURE 2. Penalized splines using generalized additive mixed models of total 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) vs. log pregnanediol-3-glucuronide (PdG), and log estrone conjugates (E_1C), in 287 female textile workers who did not smoke, Anhui, China, 1996–1998. Log PdG and E_1C on the *y*-axes are shown as the deviation from the mean; dashed lines, 95 percent confidence intervals. Represented are a total of 510 cycles, all phases. Nineteen indicator variables were included in models to adjust for each day in the 20-day window around ovulation. Additional covariates in the adjusted models were age, age squared, body mass index, body mass index squared, education (high school/middle school), passive smoke exposure (yes/no), shift work (yes/no), stress (low, moderate, high levels), noise exposure (low, moderate, high levels), and dust exposure (low, moderate, high levels).

during the luteal phase. Median *p*,*p*′-DDE levels in this population were 2,355 ng/g of lipid. The Windham et al. study did not find significant inverse associations of *p*,*p*′-DDE or *p*,*p*′-DDT with estrogen metabolite levels, but the study's power was limited by a small sample size. Our replication of Windham et al.'s unique DDT–luteal PdG association represents important progress in understanding the action of hormonally active agents such as DDT and their potential relation to human reproductive health.

The biologic basis whereby DDT forms might affect progesterone and estrogen metabolite profiles is unknown but potentially involves multiple mechanisms, including changes in the biosynthesis and metabolism of sex steroids as well as receptor-mediated effects. For example, *p*,*p*′-DDE has been shown to bind to the progesterone receptor (22) and to inhibit progesterone-induced reporter gene activity in yeast and human breast cancer cells (23). In addition, *o*,*p*′-DDT and *o*,*p*′-DDE bind the estrogen receptor and can inhibit binding of endogenous estradiol (24). It has been established that DDT and its isomers and metabolites can induce hepatic microsomal monooxygenases including enzymes involved in steroid hormone metabolism (25). *o*,*p*′-DDE and *p*,*p*′-DDE have been shown to inhibit ovarian progesterone synthesis (26), whereas *o*,*p*′-DDE induces enzymes involved in estrogen synthesis (27).

A limitation of our study is that menstrual cycles with different outcomes (nonconception, early pregnancy loss, or clinical pregnancy) complicated our ability to appropriately model the independent effects of DDT isomers and metabolites on E_1C and PdG levels. The causal connection between cycle outcomes and these hormones is plausibly in both directions; for example, hormones are causally necessary for initiation and maintenance of pregnancy, but conception and early losses also cause changes in hormone levels. We conducted analyses both with and without conditioning on cycle outcomes and found that our results were unchanged. However, if causal pathways between hormones and cycle outcomes exist simultaneously in both directions, there is no way to model the independent causal association between DDT and hormones. The stability of our results under several causal assumptions is reassuring, but a more ideal population for this analysis would have had only one cycle outcome.

Another potential limitation of this study is use of a single baseline DDT measure. However, in this study population, baseline serum DDT levels are likely to reflect long-term accumulation. Changes in serum total DDT over the 2-year study observation period are possible but would likely be small. In addition, serum DDT was measured in nonfasting samples, and it is uncertain what impact lack of adjustment for serum lipids may have had on our findings. In general, serum DDT levels will be overestimated in individuals with higher, compared with lower, serum lipid levels when levels are expressed on a wet-weight basis. However, it is unlikely that fasting status at baseline blood draw would have correlated with subsequent urinary PdG and E_1C production across multiple menstrual cycles. Therefore, this is a potential source of random measurement error and null bias.

The study's strengths include the relatively homogeneous study population, which limits the potential for confounding by sociodemographics or reproductive history. Participants

TABLE 3. Adjusted* linear regression models of serum total DDT†, isomers, and metabolites (per 10-ng/g increase) modeled individually and daily urinary log(PdG†) and log(E_1C†) stratified by menstrual cycle phases in 287 textile workers who did not smoke, Anhui, China, 1996–1998

	Log (PdG)			Log (E_1C)		
	β	SE†	Two-sided *p* value	β	SE	Two-sided *p* value
Follicular (days −9 to −2)‡						
Sample size (days)	3,199			3,152		
Total DDT	−0.06	0.03	0.07	−0.01	0.02	0.49
p,p′-DDT	−0.56	0.45	0.21	−0.15	0.29	0.61
p,p′-DDE†	−0.06	0.03	0.06	−0.02	0.02	0.49
p,p′-DDD†	−8.88	4.12	0.03	−3.58	2.26	0.11
o,p′-DDT	−1.11	3.24	0.73	−2.04	2.97	0.49
o,p′-DDE	−8.39	4.65	0.07	−5.00	3.29	0.13
Periovulation (days −1 to 1)						
Sample size (days)	1,337			1,337		
Total DDT	−0.06	0.03	0.04	−0.04	0.02	0.02
p,p′-DDT	−0.54	0.36	0.13	−0.43	0.20	0.03
p,p′-DDE	−0.06	0.03	0.05	−0.05	0.02	0.03
p,p′-DDD	−10.71	4.13	0.01	−5.33	2.27	0.02
o,p′-DDT	−1.51	2.63	0.57	−4.99	2.18	0.02
o,p'-DDE	−9.52	5.33	0.07	−8.84	3.42	0.01
Luteal (days 2 to 10)						
Sample size (days)	3,927			3,886		
Total DDT	−0.05	0.02	0.03	−0.04	0.02	0.04
p,p′-DDT	−0.57	0.28	0.04	−0.31	0.26	0.24
p,p′-DDE	−0.06	0.03	0.03	−0.05	0.02	0.04
p,p′-DDD	−6.53	3.10	0.04	−5.33	3.11	0.09
o,p′-DDT	−3.11	2.38	0.19	−4.04	2.71	0.14
o,p′-DDE	−13.42	3.57	<0.01	−8.15	3.37	0.02

* Nineteen indicator variables were included in models to adjust for each day in the observed 20-day window around ovulation. Standard errors were estimated to accommodate correlations in hormone concentrations in multiple urine samples from the same woman. Covariates in adjusted models included age, age squared, body mass index, body mass index squared, education (high school/middle school), passive smoke exposure (yes/no), shift work (yes/no), stress (low, moderate, high level), noise exposure (low, moderate, high level), and dust exposure (low, moderate, high level). The estimated parameters for crude models (not shown) were similar to those for adjusted models.

† DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; PdG; pregnanediol-3-glucuronide; E_1C, estrone conjugates; SE, standard error; DDE, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene; DDD, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane.

‡ Day of ovulation was designated as day 0.

were young, healthy, nonsmoking, and nulliparous. None was using exogenous hormones or other forms of birth control. We were able to obtain daily urine samples for assessment of hormone metabolite levels and detection of preclinical pregnancy, thereby benefiting from biomarkers of hormone variability throughout the menstrual cycle. Study participants had very low-level exposure to other organochlorines, with a mean sum of eight prevalent polychlorinated biphenyls of 0.15 (standard deviation, 0.06) ng/g of serum (data not shown).

Ours is among the first and largest population-based studies to evaluate the relation of DDT with a biomarker of sex steroid levels in reproductive-age women. We found consistent associations between DDT exposure and suppressed progesterone and estrogen urine metabolites at critical times in the menstrual cycle. The most consistent effects were in the luteal phase for progesterone metabolites and in the periovulation phase for estrogen metabolites. These results support the potential for DDT to be associated with decrements in progesterone and estrogen levels at times during

the menstrual cycle that are critical for ovulation and early pregnancy maintenance. This finding represents important progress in understanding hormone pathways that DDT may disrupt to adversely affect human female reproduction.

ACKNOWLEDGMENTS

This study was supported in part by grants 1R01 HD32505 from the National Institute of Child Health and Human Development; 1R01 ES08337, ES-00002, P01 ES06198, KO1 ES10959, and 1R01 ES11682 from the National Institute of Environmental Health Sciences; and 20-FY98-0701 and 20-FY02-56 from the March of Dimes Birth Defects Foundation.

Conflict of interest: none declared.

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