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Original Contribution

Preconception Serum DDT and Pregnancy Loss: A Prospective Study Using a Biomarker of Pregnancy

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Previous studies of pregnancy losses and 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) were limited because they did not include losses prior to clinical detection of pregnancy and because exposures were measured after the pregnancies of interest. The authors examined the association of preconception serum total DDT (sum of DDT isomers and metabolites) concentration and subsequent pregnancy losses in 388 newly married, nonsmoking, female textile workers in China between 1996 and 1998. Upon stopping contraception, subjects provided daily urine specimens and records of vaginal bleeding for up to 1 year or until clinical pregnancy. Daily urinary human chorionic gonadotropin was assayed to detect conception and early pregnancy losses, and pregnancies were followed to detect clinical spontaneous abortions. There were 128 (26%) early pregnancy losses in 500 conceptions and 36 (10%) spontaneous abortions in 372 clinical pregnancies. Subjects were grouped in tertiles by preconception serum total DDT concentration (group 1: 5.5–22.9 ng/g; group 2: 23.0–36.5 ng/g; group 3: 36.6–113.3 ng/g). Compared with group 1, group 2 had adjusted relative odds of early pregnancy losses of 1.23 (95% confidence interval (CI): 0.72, 2.10), and group 3 had adjusted odds of 2.12 (95% CI: 1.26, 3.57). The relative odds of early pregnancy losses associated with a 10-ng/g increase in serum total DDT were 1.17 (95% CI: 1.05, 1.29). The small number of spontaneous abortions following clinical detection of pregnancy were not associated with serum total DDT. In this population, there was a positive, monotonic, exposure-response association between preconception serum total DDT and the risk of subsequent early pregnancy losses.

abortion, spontaneous; chorionic gonadotropin; DDT; dichlorodiphenyldichloroethane; dichlorodiphenyl dichloroethylene; hormone antagonists

Abbreviations: CI, confidence interval; 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; hCG, human chorionic gonadotropin; OR, odds ratio; SD, standard deviation.

Editor's note: *A related article appears on page 717, and an invited commentary on these two articles is published on page 726.*

1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) is a synthetic pesticide that was widely used in agricultural and domestic settings during the 20th century. DDT breaks

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down in the environment and is metabolized to 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE) and 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (DDD). Because of concerns about its biologic persistence and potentially harmful effects on humans and animals, the US Environmental Protection Agency banned the use of DDT in 1972 except in cases of public health emergencies (1). By the 1980s, the use of DDT was banned in most developed countries; however, DDT is still used today in many countries where malaria is a public health problem (2). China banned agricultural use of DDT in 1984, but use of DDT for malaria control continued in some regions after the ban (3).

In animal models, increased fetal resorption or abortion has been associated with exposure to DDT (4, 5). A number of epidemiologic studies have investigated the association of spontaneous abortions in humans and DDE, the principle metabolite of DDT. Longnecker et al. (6) recently reported that the odds of fetal losses in previous pregnancies were positively associated with serum DDE concentrations in pregnant women who were enrolled in a prospective study between 1959 and 1965 in the United States. Several case-control studies reported positive associations between DDE concentrations and spontaneous abortion in populations where the DDE concentrations were relatively higher (3, 7), but other studies in populations where the concentrations were lower found no such associations (8–10).

Previous studies were limited because they measured DDT and/or its metabolites in blood or sera that were collected after the pregnancies of interest. Because breastfeeding decreases a woman's body burden of DDT and its metabolites (11), the average concentrations in sera from women who had previous livebirths (and breastfeeding) could be lower than what they would have been if sera had been collected prior to or during pregnancy (before breastfeeding). The potential for differential breastfeeding history among those with clinical spontaneous abortion compared with term deliveries could confound the associations between serum DDT levels and spontaneous abortions and might be difficult to control for adequately.

Furthermore, approximately one third of pregnancies end in loss, and two thirds of losses occur prior to clinical detection of pregnancy (12, 13). Thus, not accounting for pregnancy losses prior to clinical detection of pregnancy might diminish the power to detect associations between environmental exposures and pregnancy losses. Investigating all pregnancy losses, including those that occur prior to clinical diagnosis of pregnancy, requires prospective observation beginning prior to conception, extensive collection of biologic samples throughout the observed menstrual cycles, and utilization of a sensitive biomarker of pregnancy, such as human chorionic gonadotropin (hCG), that can detect pregnancy soon after conception. We investigated the effect of DDT on pregnancy losses in a prospective cohort of women in Anhui, China, for whom preconception serum samples were available for DDT analyses. We report the associations of preconception maternal serum DDT concentration, along with its metabolites, and subsequent pregnancy losses that occurred both before and after clinical detection of pregnancy.

MATERIALS AND METHODS

Study population

This is part of a prospective study of reproductive health conducted from 1996 to 1998 in textile mills in Anhui, China. The institutional review boards of the Harvard School of Public Health, the Children's Memorial Hospital, and collaborating Chinese institutions approved our study protocols. We obtained written, informed consent from each woman and her husband.

We previously reported a detailed description of the study population and data collection methods (13). Briefly, the eligibility criteria for women were as follows: 1) full-time employment, 2) aged 20–34 years, 3) newly married, and 4) had obtained permission to have a child. All the women were nulliparous. Women were excluded if they 1) were already pregnant before enrollment, 2) had tried unsuccessfully to get pregnant for 1 or more years at any time in the past, or 3) planned to quit/change jobs or move out of the city over the 1-year course of follow-up.

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 452 enrolled women from this analysis because they did not collect daily urine ($n = 121$), did not begin collecting urine in the first cycle after stopping contraception ($n = 53$), never stopped using contraception ($n = 95$), became pregnant due to contraceptive failure ($n = 78$), were lost to follow-up ($n = 8$), withdrew shortly after enrollment ($n = 27$), had inadequate urine or diary data ($n = 34$), did not have baseline data about the husband's smoking ($n = 8$), drank alcohol ($n = 1$), were current smokers ($n = 2$), reported occupational exposures to toxicants ($n = 5$), or did not conceive and so were not at risk for pregnancy loss ($n = 20$). Of the remaining 509 women, serum DDT concentrations were available for 388 women. Insufficient serum volume remained on the balance of samples due to previous study analyses. Sufficiency of sample volume was not associated with odds of pregnancy losses and was unlikely to have been associated with DDT concentration. The characteristics of the women excluded from this analysis were similar to those who were included. This report includes 388 women who began recording diary entries and collecting daily urine immediately after stopping contraceptive use, conceived, and had adequate DDT, diary, and hCG data. All of the women included in our analysis had never been smokers and neither drank alcohol nor reported exposures to known occupational toxicants in the textile mills.

Exposure assessment

Each subject provided a nonfasting blood sample as part of the baseline evaluation, which occurred prior to when she stopped using contraception with the intent to become pregnant. Serum fractions were frozen at −20°C until extraction. Serum samples were analyzed by the Harvard School of Public Health Organic Chemistry Laboratory (Boston, Massachusetts) for *p,p′* and *o,p′* isomers of DDT, DDE, and

TABLE 1. Distributions and correlation of preconception serum concentrations of total DDT* and isomers in 388 nonsmoking, nulliparous, female textile workers in Anhui, China, 1996–1998

	Minimum	Quartile 1	Median	Quartile 3	Maximum
Total DDT (ng/g)	5.52	19.9	27.9	42.3	113.3
p,p′-DDT (ng/g)	0.37	0.96	1.42	2.18	13.12
o,p′-DDT (ng/g)	0.04	0.12	0.16	0.24	1.49
p,p′-DDE* (ng/g)	4.76	18.31	26.24	39.53	97.54
o,p′-DDE (ng/g)	0.03	0.06	0.09	0.12	1.07
p,p′-DDD* (ng/g)	0.05	0.15	0.21	0.29	0.96
	Spearman's correlation coefficients				
	p,p′-DDT	*o,p′*-DDT	*p,p′*-DDE	*o,p′*-DDE	*p,p′*-DDD
Total DDT	0.70	0.55	>0.99	0.45	0.55
p,p′-DDT		0.64	0.67	0.39	0.79
o,p′-DDT			0.53	0.75	0.53
p,p′-DDE				0.44	0.53
o,p′-DDE					0.30

* DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; DDE, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene; DDD, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane.

DDD. Details of the laboratory analytical methods and quality control procedures are reported elsewhere (14). Briefly, gas chromatographic analyses of serum extracts used electron-capture detection and capillary columns of different polarity for primary and confirmatory analyses of all samples. Quantification was based on the response factor of each analyte relative to an internal standard. Final concentrations of analyte were reported in nanograms per gram of serum after subtracting the amount of analyte measured in the procedural blank. The lipid content of the serum was not measured because serum volume was insufficient. The analyst was blind to the pregnancy loss status of the samples.

As shown in table 1, among the DDT isomers and metabolites that were measured in this study, *p,p′*-DDE had the highest concentration and, on average, accounted for 92 percent of the mass of the DDT isomers and metabolites measured in serum (range: 76–98 percent). The Spearman correlation coefficient for total DDT and *p,p′*-DDE was greater than 0.99. *p,p′*-DDT had the second highest concentration and, on average, accounted for 6 percent of the mass (range: 2–19 percent). Summed together, *p,p′*-DDE and *p,p′*-DDT accounted, on average, for 98 percent of the mass of the DDT isomers and metabolites measured in serum (range: 93–100 percent). Because *p,p′*-DDE and *p,p′*-DDT were highly correlated (Spearman's correlation coefficient = 0.67), we conducted our analyses 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 also conducted our analyses modeling *p,p′*-DDE and *p,p′*-DDT individually and together.

Laboratory assay of urinary hCG

We analyzed urine specimens for hCG using the immunoradiometric assay developed by O'Conner et al. (15) that included a combination of capture antibodies for hCG free beta-subunit and hCG beta-core fragment (B204 antibody) and the intact hCG molecule (B109 antibody). This assay was highly sensitive and specific. The lowest hCG concentration detectable by the assay was 0.01 ng/ml (1 mIU = 0.2 ng) (16). The cross-reaction of the assay with either intact luteinizing hormone or luteinizing hormone free subunit was less than 1 percent. We analyzed and tested all urine specimens from each woman during a single run of the assay. We assayed each urine specimen in duplicate during the window from −10 to 5 days of a menstrual cycle and repeated the assays if the discrepancy between duplicates was greater than threefold. For pairs of duplicate assays with less than a threefold difference, we used the geometric mean of the two in our analyses. We used the method of Jaffe, as described by Husdan and Rapoport (17), to measure urine creatinine levels and normalized hCG values to creatinine to adjust for urine concentration. As reference values, we determined levels of hCG from 67 nonconception cycles of 37 female controls who were married but using contraception ($n = 4$), not married ($n = 23$), or married but not cohabitating with their husbands ($n = 10$) (13).

Major outcomes and method of evaluation

There were five major outcomes of this investigation.

1. *Conception*: a conception detected by a highly sensitive and specific urinary hCG assay as described in a previous report (13). To distinguish normal variation from a true hCG rise due to conception and to address missing hCG values, we used Bayesian methods (18, 19) to model daily conception status among all the female subjects including female controls who did not conceive. We showed that this model was 100 percent sensitive and

specific for those cycles in which the conception status was observable; that is, the probability of conception was 0.0 in all control cycles and 1.0 in all cycles with conception leading to clinical pregnancy (13).
2. *Clinical pregnancy*: any pregnancy that lasted 6 weeks (42 days) or more after the onset of the last menstrual period and was confirmed by hCG assay.
3. *Early pregnancy loss*: pregnancy loss (detected by urinary hCG assay, see conception definition above) occurring less than 6 weeks (42 days) after the onset of the last menstrual period.
4. *Clinical spontaneous abortion*: pregnancy loss occurring 6 weeks (42 days) or more but no later than 20 weeks after the onset of the last menstrual period.
5. *Total pregnancy loss*: including both early pregnancy loss and clinical spontaneous abortion as define above.

Statistical methods

We grouped study women into low, medium, and high tertiles based on preconception serum concentrations of total DDT. We described important epidemiologic characteristics of subjects in each tertile using means and frequencies.

We used logistic regression to investigate the odds of total pregnancy losses and early pregnancy losses in the medium and high serum total DDT tertiles relative to the low tertile. We conducted a trend test using a single variable that was coded with the median value of the total DDT tertile to which each subject was assigned. We also estimated the relative odds of total pregnancy losses and early pregnancy losses associated with a 10-ng/g increase in serum total DDT. Because we prospectively observed study women for up to 1 year until they achieved a clinical pregnancy, women who had early pregnancy losses prior to clinical pregnancy remained in our cohort and might have had more than one observed conception. We first examined the logistic regression models for total and early pregnancy losses using only the first observed conception. We then repeated the analyses using all observed conceptions and estimated standard errors by use of generalized estimation equations to accommodate correlations in pregnancy losses among conceptions from the same women (20).

Clinical spontaneous abortion, by definition, can occur only after clinical detection of pregnancy. We therefore limited our analysis to study women who achieved clinical pregnancy, and we investigated the relative odds of clinical spontaneous abortion in the total DDT tertiles, conducted a trend test, and estimated the relative odds associated with a 10-ng/g increase in serum total DDT. According to the design of our study, there was no more than one clinical pregnancy for any subject.

We presented the results of all models with and without adjustment for age (tertiles), education less than high school (binary), body mass index less than 19 (binary), occupational exposures to dust (binary) or noise (binary), moderate or high perceived life stress (binary), habit of tea drinking (binary), and exposure to passive smoking from husband (binary).

RESULTS

Table 2 shows the characteristics of the subjects in each tertile of serum total DDT. Subjects in each tertile had similar height, weight, and body mass index, but age increased with increasing serum DDT. The prevalences of occupational exposures to dust and noise, perceived life stress, and tea drinking were similar among tertiles. The prevalence of smoking by husbands was lowest in the lowest serum DDT group.

Table 3 shows total pregnancy losses by serum total DDT group. By analysis of all observed conceptions for each subject, the prevalence of total pregnancy losses was highest in the high serum DDT group. Relative to the low serum DDT group, the adjusted odds of total pregnancy loss in the medium DDT group were 1.22 (95 percent confidence interval (CI): 0.73, 2.04) and, for the high DDT group, 2.01 (95 percent CI: 1.23, 3.28). The adjusted, two-sided p value for ordinal trend was 0.004. The adjusted relative odds of total pregnancy losses for a 10-ng/g increase in serum DDT were 1.17 (95 percent CI: 1.06, 1.30). The results were similar when only the first conception was analyzed.

Table 4 shows early pregnancy losses by serum total DDT group. By analysis of all observed conceptions for each subject, the prevalence of early pregnancy losses was highest in the high serum DDT group. Relative to the low serum DDT group, the odds of early pregnancy loss in the medium DDT group were 1.23 (95 percent CI: 0.72, 2.10) and, for the high DDT group, 2.12 (95 percent CI: 1.26, 3.57) after adjustment for important covariates. The adjusted, two-sided p value for ordinal trend was 0.004. The relative odds of early pregnancy losses for a 10-ng/g increase in serum DDT were 1.17 (95 percent CI: 1.05, 1.29) after adjustment for important covariates. The results were similar when only the first conception was analyzed.

Table 5 shows clinical spontaneous abortions by serum total DDT group. In this study, only one clinical pregnancy was observed for each subject. Analyzing only clinical pregnancies, there were only 12 clinical spontaneous abortions in each serum DDT group. The estimate of the adjusted odds of clinical spontaneous abortion in the high serum DDT tertile relative to the low tertile was 1.28. However, the confidence interval contained one (95 percent CI: 0.53, 3.10).

We conducted an additional analysis to determine if modeling p,p'-DDE and p,p'-DDT together could identify which was independently associated with total pregnancy losses, early pregnancy losses, or clinical spontaneous abortions despite the high correlation between them. When modeled individually with adjustment for important covariates, both were associated with total pregnancy losses in the first observed conception: p,p'-DDE, 10 ng/g (odds ratio (OR) = 1.19, 95 percent CI: 1.04, 1.36), and p,p'-DDT, 1 ng/g (OR = 1.21, 95 percent CI: 1.02, 1.44). When modeled together, the magnitudes of the estimated associations with total pregnancy losses were similar, but the standard errors were large: p,p'-DDE, 10 ng/g (OR = 1.15, 95 percent CI: 0.97, 1.36), and p,p'-DDT, 1 ng/g (OR = 1.08, 95 percent CI: 0.87, 1.35). A similar pattern emerged for early pregnancy losses when modeled individually: p,p'-DDE, 10 ng/g (OR = 1.16, 95 percent CI: 1.00, 1.33), and p,p'-DDT,

TABLE 2. Characteristics of 388 nonsmoking, nulliparous, female textile workers in Anhui, China, by preconception serum total DDT* concentration, 1996–1998†

	Preconception serum total DDT concentration			Two-sided *p* value‡
	Low	Medium	High	
No. of women	130	129	129	
Serum total DDT, ng/g (mean (SD*))	15.8 (4.7)	28.7 (3.8)	52.8 (14.2)	<0.001
Age, years (mean (SD))	24.6 (1.3)	24.9 (1.5)	25.3 (1.8)	0.001
Height, m (mean (SD))	1.57 (0.05)	1.58 (0.05)	1.57 (0.05)	0.969
Weight, kg (mean (SD))	49.7 (5.3)	49.4 (5.7)	48.7 (6.2)	0.340
Body mass index, kg/m^2 (mean (SD))	20.0 (1.8)	19.9 (2.1)	19.6 (2.0)	0.243
Education (no. (%))				
Elementary	5 (4)	1 (1)	0 (0)	0.229§
Middle school	86 (66)	77 (60)	80 (62)	
High school	38 (29)	49 (38)	48 (37)	
College or above	1 (1)	2 (2)	1 (1)	
Dust exposure (no. (%))	122 (94)	126 (98)	125 (97)	0.240
Noise exposure (no. (%))	112 (86)	118 (92)	117 (91)	0.322
Husband smokes (no. (%))	63 (49)	83 (64)	80 (62)	0.020
Perceived stress of living (no. (%))				
None or light	86 (66)	78 (61)	73 (57)	0.283¶
Moderate	39 (30)	48 (37)	53 (41)	
High	5 (4)	3 (2)	3 (2)	
Tea drinking (no. (%))	53 (41)	54 (42)	69 (54)	0.075

* Total DDT, sum of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane isomers and metabolites; SD, standard deviation.
† None of the subjects smoked or drank alcohol.
‡ Analysis of variance for means; chi-square test for frequencies.
§ Chi-square test for less than high school versus high school or above.
¶ Chi-square test for none or light versus moderate or high stress.

1 ng/g (OR = 1.19, 95 percent CI: 0.99, 1.42); or together: *p,p′*-DDE, 10 ng/g (OR = 1.10, 95 percent CI: 0.92, 1.32), and *p,p′*-DDT, 1 ng/g (OR = 1.10, 95 percent CI: 0.87, 1.39). Neither *p,p′*-DDE nor *p,p′*-DDT was associated with the small number of clinical spontaneous abortions modeled either individually or together (data not shown).

DISCUSSION

A major advantage of this study was that we recruited nulliparous women soon after marriage who planned to become pregnant over the course of the study. We therefore were able to collect preconception blood samples and to prospectively ascertain conception and clinical pregnancy. Because none of our subjects had ever given birth prior to recruitment, their serum concentrations of total DDT had not been decreased by prior history of breastfeeding. The average time between collection of blood samples and the beginning of the menstrual cycle in which conception first occurred was 163 (standard deviation: 159) days. Because DDT isomers and metabolites are persistent, our exposure assessment should have accurately reflected the relative body burden of DDT isomers and metabolites during the periconception period. Furthermore, we used a highly sensitive and specific hCG assay to detect conceptions and early pregnancy losses that occurred prior to clinical detection of pregnancy. Additionally, our subjects were a homogeneous cohort of young women who neither smoked nor drank alcohol or coffee.

We found that, relative to women in the lowest tertile of serum total DDT concentration, the odds of early pregnancy losses were increased among those in the highest tertile and that there was a linear trend of increasing odds of early pregnancy losses with increasing serum total DDT concentration. Our results support a growing number of epidemiologic studies showing that exposure to DDT and/or its metabolites is associated with increased risk of fetal losses in humans. Longnecker et al. (6) found that serum DDE concentrations were associated with differing odds of spontaneous abortions in previous pregnancies among women recruited in the United States between 1959 and 1965. Relative to the odds of women whose serum concentrations of DDE were less than 15 μg/liter, the adjusted odds ratios of fetal loss by DDE categories were as follows: 15–29 μg/liter, 1.1 (95 percent CI: 0.9, 1.5); 30–44 μg/liter, 1.4 (95 percent CI: 1.0, 1.9); 45–59 μg/liter, 1.6 (95 percent CI: 1.1, 2.4); and ≥60 μg/liter, 1.2 (95 percent CI: 0.7, 1.9). In India, Saxena et al. (7) found higher *p,p′*-DDE concentrations in maternal blood from 10 cases of spontaneous abortion (163.8

TABLE 3. Relative odds of total pregnancy losses by preconception serum total DDT* concentration in first conception and all conceptions for 388 nonsmoking, nulliparous, female textile workers in Anhui, China, 1996–1998

	No. of women	No. of conceptions	Losses		Crude		Adjusted†	
			No.	%	Odds ratio (95% confidence interval)	Two-sided *p* value	Odds ratio (95% confidence interval)	Two-sided *p* value
First conception								
Serum total DDT concentration								
Low	130	130	34	26	Referent		Referent	
Medium	129	129	36	28	1.09 (0.63, 1.89)	0.751	1.17 (0.67, 2.06)	0.583
High	129	129	47	36	1.62 (0.95, 2.75)	0.075	1.90 (1.09, 3.33)	0.024
p_{trend}						0.060		0.018
Linear total DDT (per 10 ng/g)	388	388	117	30	1.14 (1.01, 1.29)	0.031	1.18 (1.04, 1.34)	0.009
All conceptions‡								
Serum total DDT concentration								
Low	130	155	41	26	Referent		Referent	
Medium	129	165	50	30	1.21 (0.73, 2.02)	0.467	1.22 (0.73, 2.04)	0.438
High	129	180	71	39	1.81 (1.11, 2.94)	0.017	2.01 (1.23, 3.28)	0.005
p_{trend}						0.013		0.004
Linear total DDT (per 10 ng/g)	388	500	162	32	1.14 (1.03, 1.26)	0.008	1.17 (1.06, 1.30)	0.002

* Total DDT, sum of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane isomers and metabolites.

† Models adjusted for maternal age, education, body mass index, perceived life stress, tea drinking habit, occupational exposures to dust and noise, and husband's smoking.

‡ Standard errors for both crude and adjusted models estimated to accommodate correlations in pregnancy losses among conceptions from the same woman.

TABLE 4. Relative odds of early pregnancy losses by preconception serum total DDT* concentration in first conception and all conceptions for 388 nonsmoking, nulliparous, female textile workers in Anhui, China, 1996–1998

	No. of women	No. of conceptions	Early pregnancy loss		Crude		Adjusted†	
			No.	%	Odds ratio (95% confidence interval)	Two-sided *p* value	Odds ratio (95% confidence interval)	Two-sided *p* value
First conception								
Serum total DDT concentration								
Low	130	130	26	20	Referent		Referent	
Medium	129	129	27	21	1.06 (0.58, 1.94)	0.853	1.07 (0.58, 1.99)	0.824
High	129	129	36	28	1.55 (0.87, 2.76)	0.137	1.71 (0.93, 3.12)	0.082
p_{trend}						0.111		0.061
Linear total DDT (per 10 ng/g)	388	388	89	23	1.12 (0.99, 1.28)	0.077	1.15 (1.01, 1.31)	0.041
All conceptions‡								
Serum total DDT concentration								
Low	130	155	30	19	Referent		Referent	
Medium	129	165	39	24	1.29 (0.74, 2.24)	0.368	1.23 (0.72, 2.10)	0.450
High	129	180	59	33	2.03 (1.20, 3.44)	0.008	2.12 (1.26, 3.57)	0.005
p_{trend}						0.007		0.004
Linear total DDT (per 10 ng/g)	388	500	127	25	1.15 (1.04, 1.27)	0.007	1.17 (1.05, 1.29)	0.004

* Total DDT, sum of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane isomers and metabolites.

† Models adjusted for maternal age, education, body mass index, perceived life stress, tea drinking habit, occupational exposures to dust and noise, and husband's smoking.

‡ Standard errors for both crude and adjusted models estimated to accommodate correlations in pregnancy losses among conceptions from the same woman.

TABLE 5. Relative odds of clinical spontaneous abortion by preconception serum total DDT* concentration in first clinical pregnancy for 388 nonsmoking, nulliparous, female textile workers in Anhui, China, 1996–1998

	No. of women	No. of clinical pregnancies	Clinical spontaneous abortion		Crude		Adjusted†	
			No.	%	Odds ratio (95% confidence interval)	Two-sided *p* value	Odds ratio (95% confidence interval)	Two-sided *p* value
Serum total DDT concentration								
Low	125	125	12	10	Referent		Referent	
Medium	126	126	12	10	0.99 (0.43, 2.30)	0.984	1.22 (0.51, 2.92)	0.651
High	121	121	12	10	1.04 (0.45, 2.41)	0.933	1.28 (0.53, 3.10)	0.582
p_{trend}						0.925		0.606
Linear total DDT (per 10 ng/g)	372	372	36	10	1.05 (0.87, 1.26)	0.629	1.10 (0.90, 1.34)	0.345

* Total DDT, sum of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane isomers and metabolites.

† Models adjusted for maternal age, education, body mass index, perceived life stress, tea drinking habit, occupational exposures to dust and noise, and husband's smoking.

(standard deviation (SD): 105.2) ppb) compared with 25 full-term controls (12.6 (SD: 7.0) ppb). Korrick et al. (3) conducted a small case-control study using subjects selected from the same cohort used in the present analysis and found that, compared with those women who had a livebirth, women who had experienced clinical spontaneous abortion had higher serum levels of *p,p′*-DDE (22 vs. 12 ng/g) and *o,p′*-DDE (0.09 vs. 0.05 ng/g). Several studies in which DDE concentrations were lower than in the studies above did not report significant associations between blood DDE concentrations and pregnancy losses. Sugiura-Ogasawara et al. (8) did not observe an association between blood DDE from 45 Japanese women and a history of three or more consecutive first-trimester spontaneous abortions (0.70 (SD: 0.51) ppb) compared with 30 controls (0.91 (SD: 0.58) ppb). Gerhard et al. (9) found no differences in blood DDE concentrations in 89 women in Germany with recurrent miscarriages (1.24 (SD: 0.02) ng/ml) compared with controls (mean levels not reported). Leoni et al. (10) found no differences in the blood DDE concentrations of 120 Italian women with a history of miscarriage compared with 120 controls who had livebirths (5.21 (SD: 7.47) vs. 4.57 (SD: 5.60) ppb).

There are a number of possible biologic mechanisms through which maternal DDT exposure could increase the risk of pregnancy loss. However, neither laboratory nor the limited epidemiologic evidence in support of this association suggests a particular causal pathway. Longnecker et al. (6) postulated that the potential of DDT to disrupt placental cell membrane sodium channel closure and to inhibit the binding of progesterone to its receptor might be particularly relevant to DDT-associated fetal loss. A number of in vitro studies have demonstrated that DDT and its metabolites (including *p,p*-DDE, the most prevalent form in our study population) alter not only progesterone but also numerous other hormonal functions and receptor signaling pathways, including endocrine-mediated calcium handling, and via this mechanism could impair early pregnancy maintenance, thereby predisposing to loss (21). Ovarian (corpus luteal) progesterone production is critical to maintenance of early pregnancy, and both in vitro and in vivo evidence supports potential DDT-related disruption of this process by both estrogenic and nonestrogenic actions (22, 23). DDE-associated decrements in progesterone metabolites during the luteal phase of the menstrual cycle have been demonstrated in Southeast Asian immigrants in the United States (24). Furthermore, in a rabbit model, DDT has been associated with decreased serum progesterone levels in early pregnancy (25).

Although our findings are robust, we cannot eliminate the possibility that there might have been unmeasured environmental agents that were associated with serum total DDT and that were the actual agents causing the increased risk of pregnancy losses. We are also unable to determine if the associations observed were due to exposure of the mother and/or fetus during pregnancy or if maternal serum total DDT concentrations were markers of earlier exposures of the mothers in utero or during early childhood that affected their subsequent reproductive development. In addition, because of high correlations, we are unable to identify specific isomers of DDT and DDE that might be the causal agents in the association observed between total DDT and early and total pregnancy losses. It is also uncertain what impact the lack of serum lipid adjustment of total DDT levels may have had on our findings. In general, serum total DDT levels will be relatively overestimated in individuals with higher, compared with lower, serum lipid levels when concentrations are expressed on a wet weight basis (as in our study). Therefore, if serum lipids were positively associated with outcome in our analysis, this would be a potential source of bias. However, it is unlikely that fasting status at the study blood draw (a potentially important source of serum lipid variability in this setting) would correlate with the risk of fetal loss. Furthermore, in healthy reproductive aged women, there is no clear association of overall serum lipids with fetal loss (26, 27).

In conclusion, this prospective study links preconception serum total DDT concentrations to increased risk of early pregnancy loss and adds to an increasing body of literature on the adverse reproductive health effects of persistent organic pollutants.

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