Preconception serum 1,1,1-trichloro-2,2,bis(p-chlorophenyl)ethane and B-vitamin status: independent and joint effects on women’s reproductive outcomes

Parul Christian, Mei-Cheng Wang, and Xiaobin Wang

ABSTRACT

Background: Although preconception 1,1,1-trichloro-2,2, bis(p-chlorophenyl)ethane (DDT) exposure and B-vitamin deficiencies have each been shown to negatively affect human reproductive outcomes, little is known about their joint effect.

Objective: We sought to examine whether B-vitamin sufficiency protects against adverse effects of DDT on clinical pregnancy (CP) and subclinical early pregnancy loss (EPL).

Design: We measured preconception concentrations of plasma B vitamins (vitamin B-6, vitamin B-12, and folate) and serum total DDT [sum of p,p’ and o,p’ isomers of DDT and 1,1-dichloro-2,2-bis (p-chlorophenyl)ethylene] in 291 nulligravid women from Anhui, China, who were studied in 1996–1998. The women were followed prospectively from the time they stopped contraception until CP (gestational age ≥42 d) or 12 mo (whichever occurred first). EPL was identified by using daily urinary human chorionic gonadotropin. The women were categorized according to B-vitamin status (deficiency compared with sufficiency) and DDT concentration (high compared with low).

Results: Of 291 study women, a total of 385 conceptions (31% of which ended in EPL) and 265 CPs occurred. Compared with women with adequate B-vitamins and low DDT, incidence rates of CP were reduced in women with B-vitamin deficiency and a high DDT concentration (P < 0.05 for all). Most notably, in women with sufficient vitamin B-12, DDT was not associated with the incidence of CP; in contrast, in women with vitamin B-12 deficiency, high DDT was associated with a lower incidence of CP (HR: 0.44; 95% CI: 0.23, 0.84); and the test for interaction was significant (P < 0.05). The odds of EPL decreased by 45% (95% CI: 21%, 62%) for each interquartile distance increase in folate in women with high DDT concentrations, and the test for interaction was significant (P = 0.006).

Conclusions: Our results provide suggestive evidence that vitamin B-12 and folate sufficiency may help protect against adverse reproductive effects of DDT exposure. Additional studies are needed to confirm our findings.


Keywords B vitamin, clinical pregnancy, DDT, early pregnancy loss, preconception

INTRODUCTION

Difficulty conceiving is prevalent in both developed (4–17%) and developing (7–9%) countries (1). In the United States, the percentage of married women aged 15–44 y who had difficulty achieving and maintaining pregnancy increased from 8% in 1982 to 11.8% in 2002 (2, 3), and the increase cannot be completely explained by the women’s ages (4). Environmental pollutants and micronutrient deficiencies may play an important part in these population trends in human reproduction (5). 1,1,1-Trichloro-2,2,bis(p-chlorophenyl)ethane (DDT)5 was the first intentionally released chemical shown to be an endocrine...
disrupter (6, 7). DDT was a pesticide widely used in agricultural
and domestic settings from the 1940s to the 1970s. Although the
(agricultural) use of DDT was banned in most developed
countries by the 1980s and, in China, in 1984, DDT is still one
of the strategies used to reduce the transmission of malaria by
killing mosquitoes and repelling them from interior surfaces in
countries (including Ethiopia, South Africa, India, Mauritius,
Myanmar, Yemen, Uganda, Mozambique, and Swaziland) where
malaria is a public health problem (8, 9). Because of its long
biological half-life, DDT and its degradation product 1,1-dichloro-
2,2-bis(p-chlorophenyl)ethylene (DDE) are still present around the
world in the environment, food supply, and human tissues (8).

Thus far, studies on the reproductive effects of DDT have
yielded mixed results (10–12). We previously reported that
preconception serum total DDT was associated with increased
risk of early pregnancy loss (EPL) in a prospective preconception
cohort of young women with relatively high DDT concentrations
by using a highly sensitive and specific assay of daily urinary hu-
man chorionic gonadotropin (hCG) to capture conception and EPL
outcomes (13). In principle, increased risk of EPL can lengthen the
time to clinical pregnancy (CP).

Preconceptional and periconceptional B-vitamin deficiencies
are common worldwide (14) (15) and are of particular concern
because they affect both fertility (16) and the developing embryo/
fetus (17). There is growing recognition that dietary factors and
environmental exposures often co-occur via contaminated food
(from soil pesticide residues). There has also been evidence that
human health can be both positively and negatively influenced by
the interplay between dietary factors and environmental expo-
sures (5). This evidence raises the possibility that B-vitamin
status could either enhance or attenuate DDT and DDE effects.
However, to our knowledge, this hypothesis has not yet been
tested in a human population (18).

The central focus of this study was to investigate whether
effects of B vitamins and DDT are independent or whether B-
vitamin sufficiency can protect against the adverse effects of
DDT on early reproductive outcomes including CP and EPL.

SUBJECTS AND METHODS

Study population

This study used data from a prospective study of reproductive
health and rotating shift work conducted from 1996 to 1998 in
textile mills in Anhui, China (19, 20). All of the women were
newly married, nulligravid textile workers who intended to be-
come pregnant. Women were not occupationally exposed to
DDT on early reproductive outcomes including CP and EPL.

As detailed elsewhere (21), eligibility criteria included 1) full-
time employment, 2) age from 2 to 34 y, 3) newly married, and
4) the woman and her husband obtained permission to have a
child. Exclusion criteria included 1) being pregnant before
enrollment, 2) having tried unsuccessfully to get pregnant for
\( \geq 1 \) y at any time in the past, or 3) planned to quit or change jobs
or move out of the city over the 1-y follow-up period.

At baseline, each woman completed a physical examination,
blood draw, and a questionnaire that assessed demographic in-
formation, the use of contraceptives and vitamin supplements,
smoking, passive smoking, alcohol use, and reproductive history.
Beginning from the date that they stopped using any contra-
ceptive method and attempted to conceive, each woman collected
daily first-morning urine specimens (for the hCG assay) and kept
a daily diary to record sexual intercourse and vaginal bleeding
until a pregnancy was clinically confirmed or a maximum of
12 mo, whichever came first. As such, there was no unobserved
time for the time to conception analysis. All pregnancy outcomes
were recorded.

Of 1006 newly married women who were screened for the
parent study, 971 women met the enrollment criteria, and 961
women were enrolled. For this analysis, we excluded 432 en-
rolled women because they did not collect daily urine (\( n = 121 \)),
did not begin collecting urine during the first cycle after stop-
ning contraception (\( n = 53 \)), never stopped the use of contra-
ception (\( n = 95 \)), became pregnant because of 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 of husband’s smoking (\( n = 12 \)),
drank alcohol (\( n = 1 \)), or reported current smoking (\( n = 2 \) or
occupational exposure to toxicants (\( n = 5 \)). Of the remaining
525 women, serum DDT concentrations were available for 408
women in whom serum B-vitamin and folate concentrations
were available for 291 women. There were no significant dif-
fferences in demographic and exposure characteristics in the 117
women without DDT data, 117 women with DDT but without
B-vitamin data, and 291 women with DDT and B-vitamin data
(data not shown) except for education (proportions of women
with a high school education or higher were 31.6%, 48.7%, and
31.3%, respectively, for each of the 3 groups; \( P = 0.002 \)).

DDT exposure and B-vitamin assessment

At baseline, we collected nonfasting blood samples from each
participant. Serum fractions were kept at \(-20^\circ C\) until DDT was
assayed. The plasma was stored at \(-20^\circ C\) until it was shipped
don dry ice to the Harvard School of Public Health, where it
was stored at \(-70^\circ C\) before nutritional analyses. Samples were
analyzed within a few years after the completion of the study
(20, 22). Details of the laboratory analytic methods and quality-
control procedures are reported elsewhere for serum DDT
measures (22, 23) and plasma B vitamins (20).

Samples were measured for p,p'- and o,p'-isomers of
DDT, DDE, and 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene by
the Harvard School of Public Health Organic Chemistry Labo-
atory. Briefly, for primary analyses of serum extracts, we used
gas chromatography with electron capture detection, and for
confirmatory analyses of all samples, we used capillary columns
of different polarity. Final concentrations were reported as the
mean of the 2 measures. Samples were analyzed in batches
whereby each batch included a procedural blank, matrix spike
samples, and a laboratory control sample to assess interbatch
laboratory CVs. Method detection limits were <0.04 ng/g for all
DDT forms. All women had serum concentrations for DDT
and its metabolites above the limits of detection. Total DDT
equaled p,p'-DDT plus p,p'-DDE plus p,p',1,1-dichloro-2,2-
bis(p-chlorophenyl)ethylene plus o,p'-DDT plus o,p'-DDE (22, 23).
The lipid content of each serum sample was not measured because
the sample volume was insufficient.

Plasma vitamin B-6, vitamin B-12, and folate concentrations
were measured at the USDA Human Nutrition Research Center
on Aging at Tufts University (20). Plasma vitamin B-6 (as pyridoxal 5'-phosphate) was measured by using the tyrosine decarboxylase apoenzyme method. Plasma vitamin B-12 and folate were measured by using a radioimmunoassay with a commercially available kit from the BioRad Diagnostics Group. B-vitamin measurements were completed in 4 batches over an 11-mo period with from 63 to 282 samples in each batch. Typical CVs for in-house control plasma samples were <8% for folate and vitamin B-12 and <9% for vitamin B-6. Vitamin deficiency was defined as concentrations <30 nmol pyridoxal 5'-phosphate/L for vitamin B-6, <258 pmol/L for vitamin B-12, and <6.8 nmol/L for folate.

Laboratory assays of urinary hCG

Urine specimens were stored at −20°C. Urinary hCG was analyzed by the immunoradiometric assay developed by O’Connor et al. (24) by using a combination of capture antibodies for the hCG free β subunit and hCG β core fragment (B204) and the intact hCG molecule (B109) (21). This assay is highly sensitive and specific with a lowest detectable hCG concentration of 0.01 μg/L (1 μg = 5 IU) (21). The cross-reaction of the assay with either intact luteinizing hormone or luteinizing hormone free β subunit was <1%. All urine specimens from a given participant were analyzed and tested during a single run of the assay. Each urine specimen during the window from −10 to 5 d of a menstrual cycle was assayed in duplicate (the assay was repeated if discrepancies between duplicate assays were >3-fold). The geometric mean of replicates was used to summarize the results for each sample. Urine creatinine concentrations were measured according to the method of Jaffe (25). All hCG values were normalized to creatinine concentrations to adjust for the urine concentration. As a reference value, nonconceptive concentrations of hCG were determined from the 67 complete cycles of the 37 control women (21).

Major outcomes and method of evaluation

The major outcomes of this study included the time to CP and its 2 intermediate outcomes of the time to first observed conception (i.e., hCG-detected pregnancy) and EPL.

Time to CP

CP was defined as any pregnancy that lasted ≥6 wk (≥42 d) after the onset of the last menstrual period (LMP) that was confirmed by a daily hCG assay. The time to CP was defined as the number of menstrual cycles it took from the time that a woman stopped contraception and began attempts to conceive until she achieved a CP. Each woman contributed menstrual cycles to the survival analysis (see Statistical analysis) until she became clinically pregnant or a maximum of 12 mo of follow-up had occurred. Because, technically, it remains beyond our reach to detect the amount of pregnancy loss from fertilization to the time of implantation (26), we believe that time to CP may well reflect the overall effect of DDT on early reproductive outcomes including the time to fertilization, loss after fertilization occurring before implantation, EPL, and repeated occurrences of these.

For the cycle definition, the first cycle was defined as starting at the attempt to become pregnant. For the second menstrual cycle and so on, a cycle was defined as starting at the first (bleeding) day of a menstruation and continuing to the beginning of the next menstruation on the basis of the woman’s daily diary.

EPL

EPL was defined as pregnancy loss (detected by highly sensitive and specific assay of daily urinary hCG without clinical recognition) occurring ≤6 wk (42 d) after the onset of the LMP as detailed in our previous publication (21). We also defined clinical spontaneous abortion as pregnancy loss after a CP but occurring ≤20 wk after the onset of the LMP (13). Total pregnancy loss included both EPL and a clinical spontaneous abortion as previously defined.

Statistical analysis

Conception status and EPL were determined on the basis of an hCG assay. We used Bayesian methods to model daily conception status in all female subjects including control women who did not conceive. This model allowed us to calculate a probability of conception for each observed cycle. In a previous report, we showed that this model was 100% sensitive and specific for cycles with known conception status; the probability was zero for all of control cycles and 1.0 for all cycles with conception leading to CP (21).

The total DDT amount was classified as low or high by using a median split (30.7 ng/g) because there was no clinically meaningful split. Then, on the basis of DDT (low or high) and B-vitamin status (normal or deficiency), we grouped study women into 4 groups as follows: 1) low DDT, normal B vitamin, 2) low DDT, B-vitamin deficiency, 3) high DDT, normal B vitamin, and 4) high DDT, deficiency for vitamin B-6, vitamin B-12, and folate, respectively. On the basis of the number of B-vitamin deficiencies a woman displayed, we grouped study women into 6 groups as follows: low or high DDT in combination with 0, 1, or ≥2 B-vitamin deficiencies, respectively. The group with low DDT and no B-vitamin deficiencies comprised the reference group.

The primary endpoint of interest was CP. We first used the Kaplan-Meier curve to assess the cumulative incidence rate of CP across the number of menstrual cycles in the DDT and vitamin groups for vitamin B-6, vitamin B-12, and folate and the number of vitamin deficiencies. We estimated the hazard difference between each group of DDT and B-vitamin status compared with the group with low DDT, normal B vitamins by using semiparamatric additive hazard models (27). We compared the goodness of fit of Weibull parametric estimates to nonparametric (Kaplan-Meier) estimates of the cumulative distribution function (CDF) of time to CP by using probability plotting of the CDF (in an inverse-distribution scale) in the lifereg procedure of SAS software (version 9.1; SAS Institute). The 95% CIs for the CDF estimated by the Weibull parametric compared with nonparametric method largely overlapped, suggesting that the parametric models were appropriate for our data. We also plotted the log of the negative log of the survival curve \([\ln(-\ln[S(t)])]\) vs. the log of cycles \([\ln(t)]\) and the plot was close to linear.

We used Weibull regression models to examine the effect of preconception DDT and B-vitamin status and their interaction on
time to CP. We compared these Weibull models to Cox regression models and showed them to be comparable. Thus, we presented only Weibull regression results in this report. We next estimated the median survival time to CP by using Weibull regression models.

To examine the effect of preconception DDT and vitamin B-6, vitamin B-12, and folate status, respectively, on EPL in conceptions, we used logistic regression models. We also conducted models stratified by DDT and B-vitamin status, respectively, and present them as supplemental tables. B vitamins were examined as both continuous and binary (normal and deficiency) variables. We performed an additional analysis on total pregnancy loss. We also applied generalized estimation equations to accommodate for correlations in repeat conceptions from the same woman.

We tested the interaction of DDT category and B-vitamin status on the incidence of CP and odds of EPL by including a cross-product term (e.g., DDT × B vitamin) in models with indicator terms for DDT category and B-vitamin status. Similar models for evaluating the interaction with DDT were fit with B-vitamin concentration instead of binary status.

Covariates in all models included maternal age (tertiles), education less than high school (binary), BMI (in kg/m²) <9 (binary), moderate or high perceived life stress (binary), regular tea consumption (binary), occupational exposure to dust (binary) and noise (binary), and exposure to passive smoking from the husband (binary) that might be related to reproductive outcomes. This information was collected by a questionnaire interview at the time of enrollment. In addition, we adjusted for all other B vitamins in the models to estimate an independent association for each.

The majority (92%; range: 76–98%) of total DDT consisted of p,p’-DDE. p,p’-DDE and total DDT were highly correlated (Spearman’s correlation coefficient = 0.998). The second highest concentration was the p,p’-DDT, which, on average, accounted for 6% of total DDT (range: 2–19%). Because the findings were very similar for total DDT, p,p’-DDE, and p,p’-DDT, we present data only for total DDT in this report.

In our study, not all participating women were enrolled on day 1 of their menstrual cycles, which led to a variably observed length for the first cycle. To address the potential bias because of the variably observed length in the first cycle (28), we performed a sensitivity analysis on the time to CP and time to first observed conception by excluding the first cycle.

The institutional review boards of the Harvard School of Public Health, Ann & Robert H Lurie Children’s Hospital of Chicago, and collaborating Chinese institutes approved the study protocol. All women and their husbands provided written informed consent. The Johns Hopkins University Bloomberg School of Public Health Institutional Review Board approved the secondary analyses that used existing datasets.

RESULTS

Study population

This study included 291 women. Of the 291 subjects, 280 women had a total of 385 conceptions; 71 women had ≥2 conceptions (range: 2–5 conceptions), and 120 conceptions (31%) ended in EPL in 81 women (range: 1–5 EPLs/woman). There were 265 CPs, one for each woman, and 26 censored observations by the end of the 1-y study period. In all, 25 CPs ended in a clinical spontaneous abortion (mean ± SD gestational age: 10 ± 2 wk; range: 7–16 wk). For 145 total pregnancy losses in 100 women (mean: 1.45 losses/woman), 69 women had 1 loss, 21 women had 2 losses, 7 women had 3 losses, 2 women had 4 losses, and 1 woman had 5 total pregnancy losses while in the study.

Study participants were young and generally lean. The mean (±SD) age was 24.9 ± 1.5 y, and mean BMI was 19.8 ± 2.1 (Table 1). Most women (68%) had a middle-school education. More than one-half (57%) of the women reported exposure to passive smoking (husband smoking). None of the women smoked. Nearly all of the women were occupationally exposed to dust (96.6%) and noise (92.1%), and 42% of the women reported moderate to high life stress. Only ~2% of women used vitamin supplements of any kind.

The median serum total DDT was 30.7 [interquartile distance (IQR): 23.2 ng/g] (Supplemental Table 1). B-vitamin deficiencies were common in this cohort; 26.5% of women were

<table>
<thead>
<tr>
<th>Characteristics of 291 nulligravid women in Anhui, China, 1996–1998</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>24.9 ± 1.5¹</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.57 ± 0.05</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>49.1 ± 6.0</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>19.8 ± 2.1</td>
</tr>
<tr>
<td>Total DDT, ng/g</td>
<td>34.4 ± 17.9</td>
</tr>
<tr>
<td>Vitamin B-6, nmol/L</td>
<td>39.2 ± 13.8</td>
</tr>
<tr>
<td>Folate, mol/L</td>
<td>10.1 ± 4.5</td>
</tr>
<tr>
<td>Vitamin B-12, pmol/L</td>
<td>364.2 ± 129.9</td>
</tr>
<tr>
<td>Education, n (%)</td>
<td></td>
</tr>
<tr>
<td>Elementary school</td>
<td>2 (0.7)</td>
</tr>
<tr>
<td>Middle school</td>
<td>198 (68.0)</td>
</tr>
<tr>
<td>High school</td>
<td>90 (30.9)</td>
</tr>
<tr>
<td>College or above</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>Dust exposure, n (%)</td>
<td>281 (96.6)</td>
</tr>
<tr>
<td>Noise exposure, n (%)</td>
<td>268 (92.1)</td>
</tr>
<tr>
<td>Perceived stress of living, n (%)</td>
<td>169 (58.1)</td>
</tr>
<tr>
<td>None or light</td>
<td>115 (39.5)</td>
</tr>
<tr>
<td>Moderate</td>
<td>7 (2.4)</td>
</tr>
<tr>
<td>High</td>
<td></td>
</tr>
<tr>
<td>Tea drinker, n (%)</td>
<td>134 (46.1)</td>
</tr>
<tr>
<td>Husband smokes, n (%)</td>
<td>166 (57.0)</td>
</tr>
<tr>
<td>Low DDT (n = 145), n (%)</td>
<td></td>
</tr>
<tr>
<td>Vitamin B-6 concentration &lt;30 nmol/L</td>
<td>32 (22.1)</td>
</tr>
<tr>
<td>Vitamin B-12 concentration &lt;258 pmol/L</td>
<td>28 (19.3)</td>
</tr>
<tr>
<td>Folate concentration &lt;6.8 mmol/L</td>
<td>27 (18.6)</td>
</tr>
<tr>
<td>No. of B-vitamin deficiencies, n (%)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>80 (55.2)</td>
</tr>
<tr>
<td>1</td>
<td>47 (32.4)</td>
</tr>
<tr>
<td>≥2</td>
<td>18 (12.4)</td>
</tr>
<tr>
<td>High DDT (n = 146), n (%)</td>
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</tr>
<tr>
<td>Vitamin B-6 concentration &lt;30 nmol/L</td>
<td>45 (30.8)</td>
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<tr>
<td>Vitamin B-12 concentration &lt;258 pmol/L</td>
<td>20 (13.8)</td>
</tr>
<tr>
<td>Folate concentration &lt;6.8 mmol/L</td>
<td>33 (22.6)</td>
</tr>
<tr>
<td>No. of B-vitamin deficiencies, n (%)</td>
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</tr>
<tr>
<td>0</td>
<td>79 (54.5)</td>
</tr>
<tr>
<td>1</td>
<td>38 (26.2)</td>
</tr>
<tr>
<td>≥2</td>
<td>28 (19.3)</td>
</tr>
</tbody>
</table>

¹Mean ± SD (all such values).
²DDT, 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane.
vitamin B-12 deficiency, 16.6% of women were vitamin B-12 deficient, 20.6% of women were folate deficient, and 15.9% of women were deficient in ≥2 vitamins. The median (IQR) was 36.6 nmol/L (17.8 nmol/L) for vitamin B-6, 343.9 pmol/L (159.3 pmol/L) for vitamin B-12, and 9.2 nmol/L (4.8 nmol/L) for folate. The serum total DDT concentration was not correlated with plasma vitamin B-12 or B-6 concentrations ($P > 0.05$) and was weakly correlated with folate concentrations ($r = -0.12, P < 0.05$; Supplemental Table 1).

**Total DDT and B-vitamin status and CP**

Overall, the cumulative incidence of CP was lowest for women with high DDT and a deficiency in any of the 3 vitamins compared with in the other groups, in particular for vitamin B-12 (Supplemental Figure 1, Supplemental Table 2). Compared with women with adequate B-vitamins and low DDT, incidence rates of CP were reduced in women with B-vitamin deficiency and high DDT concentrations ($P < 0.05$ for all; Table 2).

In women with low serum concentrations of DDT, the incidence of CP was essentially unchanged in those with vitamin B-12 deficiency [adjusted HR (aHR): 1.17; 95% CI: 0.74, 1.86; Table 2]. In contrast, in women with high DDT, those with vitamin B-12 deficiency had a substantially reduced incidence of CP (aHR: 0.50; 95% CI: 0.29, 0.86; Supplemental Table 3; $P$-effect modification = 0.02; Table 2). With vitamin B-12 expressed on a continuous scale, the effect modification was also clear; in women with high DDT, the incidence of CP increased by 23% (95% CI: −2%, 54%) for every 159.3-pmol/L (IQR) increase in vitamin B-12; whereas in women with low DDT, the incidence of CP unexpectedly decreased by 20% (95% CI: 1%, 35%) for each IQR increase in vitamin B-12 ($P$-effect modification = 0.005). In contrast, in women with sufficient vitamin B-12, DDT was not associated with the incidence of CP (aHR: 1.03; 95% CI: 0.78, 1.36), but it was associated with the incidence of CP in women with vitamin B-12 deficiency (HR: 0.44; 95% CI: 0.23, 0.84) (Supplemental Table 4) with $P$-interaction =0.02 (Table 2).

The median time to CP (i.e., time at which one-half of the women had a CP) was greatest for women with high DDT and vitamin deficiency. For example, the median time to CP was 2.4 menstrual cycles longer in women with high DDT and vitamin B-12 deficiency than in women with low DDT and normal vitamin B-12 (5.3 compared with 2.9 cycles), and the median time to CP was <3 cycles in the other 2 groups of women. In comparison with women with low DDT and no vitamin deficiencies, the incidence of CP was reduced by 53% in women with high DDT and ≥2 vitamin deficiencies (HR: 0.47; 95% CI: 0.28, 0.77) (Table 2). After the exclusion of the first cycle, results for associations of total DDT and B-vitamin status with CP were similar to what we observed for all cycles (Supplemental Table 5).

**Table 2**

| HRs summarizing the effect of preconception DDT and B-vitamin status on incidence of clinical pregnancy in 291 women prospectively followed from preconception to clinical pregnancy or up to 12 mo (whichever occurred first)$^1$
<table>
<thead>
<tr>
<th>Vitamin B-12, pmol/L</th>
<th>Low DDT</th>
<th>High DDT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude</td>
<td>Adjusted</td>
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<tr>
<td>Vitamin B-12, pmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>2.9</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>&lt;258</td>
<td>2.7</td>
<td>1.12 (0.72, 1.73)</td>
</tr>
<tr>
<td>Linear vitamin B-12, /IQR</td>
<td>—</td>
<td>0.80 (0.65, 0.99)</td>
</tr>
<tr>
<td>Vitamin B-6, nmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>2.6</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>&lt;30</td>
<td>3.8</td>
<td>0.61 (0.40, 0.92)</td>
</tr>
<tr>
<td>Linear vitamin B-6, /IQR</td>
<td>—</td>
<td>1.22 (1.001, 1.49)</td>
</tr>
<tr>
<td>Folate, nmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>2.7</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>&lt;6.8</td>
<td>3.6</td>
<td>0.67 (0.43, 1.04)</td>
</tr>
<tr>
<td>Linear folate, /IQR</td>
<td>—</td>
<td>1.13 (0.97, 1.33)</td>
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<tr>
<td>No. of B-vitamin deficiencies</td>
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<td></td>
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<tr>
<td>0</td>
<td>2.5</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>1</td>
<td>3.5</td>
<td>0.63 (0.43, 0.92)</td>
</tr>
<tr>
<td>≥2</td>
<td>3.0</td>
<td>0.77 (0.45, 1.32)</td>
</tr>
</tbody>
</table>

$^1$Clinical pregnancy was defined as any pregnancy that lasted ≥6 wk (≥42 d) after the onset of the last menstrual period and was confirmed by using a human chorionic gonadotropin assay. The analysis was adjusted for maternal age, education, BMI, perceived life stress, tea drinking habit, occupational exposure to dust and noise, and husband smoking. The IQR was 159.3 pmol/L for vitamin B-12, 17.8 nmol/L for vitamin B-6, and 4.8 nmol/L for folate. CP, clinical pregnancy; DDT, 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane; IQR, interquartile distance.

$^2$Time at which 50% of women had a clinical pregnancy was estimated on the basis of Weibull models. Delayed CP was represented by a longer median time to CP.

$^3$Estimated in the adjusted model with DDT (low or high) and vitamin (normal or deficiency) and the interaction of the two.

$^4$Estimated in the adjusted model with DDT (low or high) and vitamin (linear term) and the interaction of the two.
Total DDT and B-vitamin status and time to first observed conception

Associations of DDT and vitamin B-12 with the time to first observed conception were similar to those for CP (Table 3, Supplemental Tables 3 and 4).

In women with high DDT, the incidence of conception increased by 47% (95% CI: 18%, 83%) for every IQD increment in vitamin B-6 (Table 3), whereas no association was shown between vitamin B-6 and the incidence of conception (aHR: 0.99; 95% CI: 0.80, 1.23) in women with low DDT (P-effect modification = 0.01). We showed no association between folate and conception incidence in both low- and high-DDT groups.

After the exclusion of the first cycle, results for associations of total DDT and B-vitamin status with the incidence of first observed conception were similar to what we observed for all cycles (Supplemental Table 6).

Total DDT and B-vitamin status and EPL

In women with low DDT, odds of EPL were unchanged with increasing plasma concentrations of folate. In contrast, in women with high DDT, odds of EPL were reduced by 45% (95% CI: 21%, 62%) for every 4.8-mol/L (IQD) increase in folate (P-effect modification = 0.006; Table 4); when folate was modeled as a binary variable (normal and deficiency), associations were NS (OR: 1.46; 95% CI: 0.73, 2.89; Supplemental Table 3)

For vitamin B-6, odds of EPL were reduced with increasing vitamin B-6 concentrations, but this result did not depend on the DDT group (P-effect modification =0.43; Table 4). Vitamin B-12 concentrations were not associated with risk of EPL in either the low- or the high-DDT group. With additional adjustment for other B-vitamins in all of the previously adjusted models, results were similar (data not shown).

We also performed an additional analysis on total pregnancy loss. The magnitude and significance of associations of preconception DDT and B-vitamin status with total pregnancy loss were similar to those for EPL (Supplemental Table 7). In 280 women who had one or more pregnancies while in the study, women with high DDT and B-vitamin deficiency had a higher proportion of ≥2 total pregnancy losses (i.e., repeated pregnancy losses; Supplemental Table 8). For example, 19.1% of women with high DDT and vitamin B-6 deficiency compared with 7.3% of women with low DDT and normal vitamin B-6 had ≥2 pregnancy losses.

**DISCUSSION**

To our knowledge, this is the first study to examine the joint or interactive effects of DDT and B-vitamin status on reproductive outcomes in a prospective cohort of preconception women who were newly married and intended to become pregnant. Our findings suggest that optimal B-vitamin status may help protect against adverse reproductive effects of DDT. Beneficial effects of B vitamins on fertility outcomes might be limited to women with high DDT exposure. We observed these associations at serum DDT concentrations that were substantially higher than those likely in the contemporaneous United States. The median serum p,p’-DDE

**TABLE 3**

HRs summarizing the effect of preconception DDT and B-vitamin status on the incidence of the first observed conception in 291 women prospectively followed from preconception to clinical pregnancy or up to 12 mo (whichever occurred first)1

<table>
<thead>
<tr>
<th>Vitamin B-12, pmol/L</th>
<th>Low DDT</th>
<th></th>
<th>High DDT</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median time to first conception, cycles</td>
<td>HR (95% CI)</td>
<td>Median time to first conception, cycles</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td></td>
<td>Crude</td>
<td>Adjusted</td>
<td>Crude</td>
<td>Adjusted</td>
</tr>
<tr>
<td>Normal</td>
<td>2.4</td>
<td>1.00 (reference)</td>
<td>1.00 (reference)</td>
<td>2.1</td>
</tr>
<tr>
<td>&lt;258</td>
<td>1.7</td>
<td>1.54 (1.02, 2.34)</td>
<td>1.78 (1.15, 2.75)</td>
<td>3.5</td>
</tr>
<tr>
<td>Linear vitamin B-12, /IQD</td>
<td>—</td>
<td>—</td>
<td>0.77 (0.63, 0.93)</td>
<td>—</td>
</tr>
<tr>
<td>Vitamin B-6, nmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>2.2</td>
<td>1.00 (reference)</td>
<td>1.00 (reference)</td>
<td>2.1</td>
</tr>
<tr>
<td>&lt;30</td>
<td>2.4</td>
<td>0.88 (0.59, 1.32)</td>
<td>0.90 (0.59, 1.40)</td>
<td>3.0</td>
</tr>
<tr>
<td>Linear vitamin B-6, /IQD</td>
<td>—</td>
<td>—</td>
<td>0.99 (0.80, 1.23)</td>
<td>—</td>
</tr>
<tr>
<td>Folate, nmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>2.1</td>
<td>1.00 (reference)</td>
<td>1.00 (reference)</td>
<td>2.3</td>
</tr>
<tr>
<td>&lt;6.8</td>
<td>2.8</td>
<td>0.68 (0.44, 1.04)</td>
<td>0.71 (0.46, 1.11)</td>
<td>2.6</td>
</tr>
<tr>
<td>Linear folate, /IQD</td>
<td>—</td>
<td>—</td>
<td>1.06 (0.90, 1.25)</td>
<td>—</td>
</tr>
<tr>
<td>No. of B-vitamin deficiencies</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2.2</td>
<td>1.00 (reference)</td>
<td>1.00 (reference)</td>
<td>2.1</td>
</tr>
<tr>
<td>1</td>
<td>2.4</td>
<td>0.86 (0.60, 1.24)</td>
<td>0.87 (0.60, 1.27)</td>
<td>2.2</td>
</tr>
<tr>
<td>≥2</td>
<td>2.2</td>
<td>1.02 (0.60, 1.72)</td>
<td>1.19 (0.68, 2.07)</td>
<td>3.2</td>
</tr>
</tbody>
</table>

1Adjusted for maternal age, education, BMI, perceived life stress, tea drinking habit, occupational exposure to dust and noise, and husband smoking. The IQD was 159.3 pmol/L for vitamin B-12, 17.8 nmol/L for vitamin B-6, and 4.8 nmol/L for folate. DDT, 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane; IQD, interquartile distance.

2Time at which 50% of women had a first conception during the follow-up period was estimated on the basis of Weibull models. Delayed first conception was represented by a longer median time to conception.

3Estimated in the adjusted model with DDT (low or high) and vitamin (normal or deficiency) and the interaction of the two.

4Estimated in the adjusted model with DDT (low or high) and vitamin (linear term) and the interaction of the two.
concentration in our study was 28.6 ng/g compared with a geometric mean of 0.59 ng/g (in women who got pregnant) in the LIFE (Longitudinal Investigation of Fertility and the Environment) study (12).

A major strength of this study was that it was carried out in a prospective preconception cohort with both DDT and B-vitamin measurements at preconception; and it offered the opportunity to examine the well-defined early reproductive outcomes of CP and EPL. In addition, the study women were young, healthy, non-smokers, and non-alcohol drinkers; hence, our results were unlikely to be biased by these known determinants of poor reproductive outcomes (5). Moreover, we used a highly sensitive and specific hCG assay and daily urine samples to determine EPL before clinically detected pregnancy, which can capture pregnancies lost after implantation. In addition, the statistical power of this study was strengthened by the relatively high and wide distribution of DDT concentrations and high prevalence of B-vitamin deficiency in the study women.

A major limitation of this study was the small sample size, although this study is the largest in this kind of studies. The small sample size may have led to possible random errors. Second, our analyses included a subset of women enrolled in the preconception cohort (291 of 961 women). It is not obvious how this limitation and confirm our study findings.

The biological mechanisms underlying the observed joint or interactive effects of DDT and B vitamins on reproductive outcomes are not well understood. Nevertheless, our study findings are biologically plausible and consistent with previous research findings. Human reproductive function is regulated by various hormones, which operate in a fine balance over the menstrual cycle and during pregnancy. Both estrogen and progesterone are required for the development of the uterine wall for implantation and sustained pregnancy. The introduction of exogenous hormones or xenobiotics that mimic hormone action or affect hormone metabolism can have profound effects on reproductive function. DDT and DDE are structurally similar to various steroid hormones including oestrogens. Both animal and in vitro studies supported the hypothesis that DDT, its metabolites, and related compounds are potentially important human reproductive toxins via endocrine disruption (29). DDT was widely used throughout the United States and Europe from the 1940s to the 1970s to control for insects on agricultural crops and insects that carry diseases such as malaria and typhus (8). DDT was banned in the United States in 1972; however, it is still used in some areas of the world primarily in malaria control programs. In animals, studies showed that DDT readily passes through the placental barrier to enter tissues of the developing fetus (30) where it produces embryotoxicity and fetotoxicity. Previous studies showed that high DDT can lead to increased risk of EPL (13). Two other cohort studies reported positive associations between DDT and DDE concentrations and risk of pregnancy loss (8). In a subset analysis of pregnant women in the U.S.
Collaborative Perinatal Project cohort, a higher maternal serum DDE concentration was associated with higher risk of reporting a previous fetal loss (31). In a previous investigation in our cohort, serum total DDT was associated with decreased progesterone and estrogen concentrations at times during the menstrual cycle that were critical for ovulation and early pregnancy maintenance (19). Low estrogen and progesterone, in turn, have been associated with nonconception, EPL, and CP (32).

B-vitamin deficiencies have been associated with adverse reproductive outcomes. A case-control study in The Netherlands showed that a diet low in vitamin B-12 during pregnancy was associated with increased risk of infant congenital heart defects (33). Vitamin B-12 deficiency affects all rapidly growing (DNA-synthesizing) tissues (34). Folate is involved in DNA replication (cell cycle) and the methylation cycle (amino acids cysteine and methionine cycle) (17). Preconceptional vitamin B-6 deficiency has been shown to decrease the probability of conception and increase risk of EPL (14). In addition, hydrophobic vitamin B-12 has been shown to be one of the best catalysts for the degradation (dechlorination) of environmental DDT (35).

Our study findings, if confirmed, may have important clinical and public health implications. The success of maternal folic acid supplementation in preventing neural tube defects worldwide is an example of how a micronutrient can affect a pregnancy outcome (36, 37). Recent evidence from epidemiologic and basic research studies suggested that the disease onset and trajectory can be both positively and negatively influenced by the interplay between dietary factors and environmental exposures (18). Although folic acid fortification of grains was implemented in 1998 in the United States, many other countries, including China, have not yet done so. B-vitamin deficiencies are common in populations without folic acid fortification. As shown in our study sample, 26.5% of women were vitamin B-6 deficient, 16.6% of women were vitamin B-12 deficient, and 20.6% of women were folate deficient.

In conclusion, in this prospective cohort study of preconception women, we showed suggestive evidence that preconception vitamin B-12 and folate sufficiency may protect against adverse effects of DDT on EPL and CP. Our study established a foundation for future investigations to further determine whether optimal B-vitamin status before conception may be a simple and cost-effective intervention to mitigate adverse reproductive effects from exposure to DDT. Our findings may also have broader implications for optimizing preconception micronutrient status to counteract adverse health effects from exposure to other ubiquitous environmental chemical pollutants.

The authors’ responsibilities were as follows—XX and XW: designed the parent cohort study; XX, SAV, and XW: conducted the parent cohort study; FO and XW: designed the current study; FO, MPL, SAV, XX, M-CW, and XW: analyzed data; FO, MPL, SAV, SJ, JZ, XX, PC, M-CW, and XW; wrote the manuscript; FO, MPL, SAV, SJ, SK, XX, M-CW, and XW: critically revised the manuscript for important intellectual content; and all authors: read and approved the final manuscript. None of the authors had any conflicts of interest pertaining to this work.

REFERENCES


