Maternal Serum Levels of Polychlorinated Biphenyls and 1,1-Dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE) and Time to Pregnancy

Dionne C. Gesink Law1, Mark A. Klebanoff2, John W. Brock3,4, David B. Dunson1, and Matthew P. Longnecker1

1 Epidemiology Branch, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, Research Triangle Park, NC.
2 Division of Epidemiology, Statistics and Prevention Research, National Institute of Child Health and Human Development, National Institutes of Health, Department of Health and Human Services, Rockville, MD.
3 National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA.
4 Chemistry Department, Warren Wilson College, Asheville, NC.

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Polychlorinated biphenyls (PCBs), once used widely in transformers and other applications, and 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE), the main metabolite of the pesticide 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT), are hormonally active agents. Changes in menstrual cycle functioning associated with PCBs and DDE, and increased odds of spontaneous abortion associated with DDE, suggest that these compounds could affect fertility. The authors investigated the association between PCB and DDE exposure and time to pregnancy by using serum levels measured in 390 pregnant women in the Collaborative Perinatal Project enrolled at 12 study centers in the United States from 1959 to 1965. They estimated adjusted fecundability odds ratios by using Cox proportional hazards modeling for discrete time data. Compared with time to pregnancy for women in the lowest exposure category (PCBs < 1.24 µg/liter, DDE < 14 µg/liter), time to pregnancy increased for women in the highest exposure category in terms of both PCBs (fecundability odds ratio for PCBs ≥ 5.00 µg/liter = 0.65, 95% confidence interval: 0.36, 1.18) and DDE (fecundability odds ratio for DDE ≥ 60 µg/liter = 0.65, 95% confidence interval: 0.32, 1.31). Overall, time to pregnancy increased with increasing serum PCB levels but was less suggestive of an association with DDE. Both trends were imprecise and attenuated when expressed on a lipid basis. Overall, evidence of an association between PCB or DDE exposure and time to pregnancy was weak and inconclusive.

Abbreviations: CI, confidence interval; DDE, 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene; DDT, 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane; OR, odds ratio; PCB, polychlorinated biphenyl.

Polychlorinated biphenyls (PCBs), used, for example, as heat exchange fluids and in transformers (1), and 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE), a metabolite of the pesticide 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT), are hormonally active agents. PCB congeners can be estrogenic, antiestrogenic, androgenic, or antiandrogenic (2), whereas DDE is antiandrogenic (3). Recently, PCBs and DDE were detected in the follicular fluid of human ovaries (4, 5). PCBs have been found to alter menstrual cycle length (6, 7), and there is evidence that DDE exposure is associated with altered ovarian function (8) and spontaneous abortion (9–11). Changes in ovarian and menstrual cycle functioning and increased odds of spontaneous abortion suggest that PCB and DDE exposure could affect fertility.

Previous animal studies have associated PCB exposure with decreased female fertility (12–15); human studies have produced mixed results (4, 16–21). In human studies,
back-calculated serum PCB levels were not significantly associated with increased time to pregnancy (20, 22, 23). Follicular PCB levels were positively correlated with pregnancy in couples undergoing in vitro fertilization (4). Few human studies have investigated the direct effects of DDE exposure on fertility, although evidence does suggest an effect. Follicular and serum DDE levels have been inversely correlated with successful in vitro fertilization (4), but there is weak evidence that time to pregnancy is increased for the spouses of DDT applicators (24). Whether PCB or DDE exposure is associated with decreased fertility in humans remains an open question.

The purpose of our study was to investigate the association of PCB and DDE exposure with time to pregnancy. We used serum PCB and DDE levels measured in a subset of pregnant women enrolled in the Collaborative Perinatal Project.

MATERIALS AND METHODS

Study subjects

The women in our study participated in the Collaborative Perinatal Project, a large prospective study of “the developmental consequences of complications in pregnancy and the perinatal period” (25, p. 185). Pregnant women were enrolled from 1959 to 1965 (before PCBs or DDE was banned in the United States) at 12 study centers across the United States, and their offspring were followed from gestation through age 7 years. Detailed information on the mothers was collected, including demographics, reproductive and gynecologic history, and recent and past medical history. Approximately 60,000 pregnancies were included in the project (26). Organochlorine levels were measured for 2,611 pregnancies that resulted in a delivery (27, 28), of which 448 were planned pregnancies (figure 1).

PCB and DDE exposure

Diet was the most likely route of PCB and DDE exposure for women in the Collaborative Perinatal Project (29). Maternal blood samples were collected during the prenatal and perinatal periods, were then frozen, and were stored for future analysis. From 1997 to 1999, third-trimester blood samples were analyzed for serum organochlorine levels for 2,611 Collaborative Perinatal Project pregnancies to assess the association between PCB and DDE levels and various outcomes in children (27). Approximately half of the subset was selected randomly (n = 1,200), and the remainder was selected according to sex-specific birth defects and neurodevelopmental test performance of the offspring. In the present study, 205 pregnancies (46 percent) were selected from the random subset.

Levels of 11 PCB congeners, DDT, and DDE were measured at the Centers for Disease Control and Prevention by using electron capture detection after solid-phase extraction, cleanup, and dual-column gas chromatography (30). The between-assay coefficients of variation were 19 percent for total PCBs at 3.54 µg/liter and 19 percent for DDE at 29 µg/liter (6, 30). PCB congeners 74, 118, 138, 153, 170, 180, and 194 were selected for measurement because they have been detected at the highest levels in human samples (31). PCB congeners 28, 52, 105, and 203 were chosen because they were neurotoxic in animal experiments (32) and are detectable in a portion of human specimens. Values for the 11 PCB congeners were summed to construct a total PCB exposure value for each study participant. Total PCBs was used for the primary analysis. PCB congeners were used for secondary analyses.

Wet-weight PCB and DDE measures were converted to lipid-basis estimates by using the total serum lipids conversion equation provided by Philips et al. (33): Total serum lipids = 2.27 × total cholesterol + triglycerides + 0.623. PCBs and DDE were analyzed statistically, expressed on a wet-weight and a lipid basis.

Time to pregnancy

Among the Collaborative Perinatal Project questions, women were asked, “Have you been trying to become pregnant?” If the response was yes, they were asked, “How long did it take you to become pregnant?” The response was recorded in months, starting at 1 month. We used this self-reported estimate as our measure of time to pregnancy.

Eight women overestimated their time to pregnancy by failing to restart their calculation of time after a spontaneous abortion. For instance, a woman who started trying to conceive in June, miscarried in August, then conceived the current pregnancy in October could report a time to pregnancy of 4 months, when in reality it took only 2 months. We corrected these estimates by recalculating time to pregnancy beginning with the date of miscarriage.

Thirty-two women started trying to become pregnant soon after giving birth or while breastfeeding, which inflated their
time-to-pregnancy estimates. Most new mothers are infertile up to 6 weeks postpartum, and menstruation during this window is often anovulatory (34, 35). On average, nonlactating women first ovulate 10 weeks postpartum, and lactating women may ovulate by 17 weeks postpartum (34). Menses will resume in 70 percent of nonlactating women by 12 weeks postpartum and will take longer to resume in lactating women (34). However, a return to menses does not necessarily mean a return to full fertility (34, 35). Shortened luteal phases (36), inadequate progesterone production (37), and elevated prolactin levels (35) reduce fertility during the puerperium. Lactational amenorrhea is an effective contraceptive for up to 6 months postpartum for women who exclusively, or nearly exclusively, breastfeed (38–40).

We did not have direct information on breastfeeding after the prior pregnancy, so we assumed that women who breastfed after the current pregnancy also breastfed after the prior pregnancy. We corrected time-to-pregnancy estimates for recent delivery or breastfeeding to reflect postpartum subfertility (2-month period of subfertility) and lactational amenorrhea (4-month period of subfertility). However, if a woman did become pregnant during this time, we did not change her time-to-pregnancy estimate.

Our final time-to-pregnancy variable included 40 estimates corrected for unaccounted miscarriage, postpartum subfertility, and lactational amenorrhea. Time-to-pregnancy estimates were missing for 21 of the 448 women trying to become pregnant.

Data analysis

We compared more fertile women (those who became pregnant in 3 months or less) with less fertile women (those who became pregnant in more than 3 months) according to risk factors known to increase time to pregnancy as a method of empirically validating our data (table 1). For PCB and DDE levels and for demographic, menstrual, and gynecologic characteristics, we also compared women who were planning to become pregnant with women who had not planned on becoming pregnant (not shown in table).

We used survival analysis to analyze the association between PCBs, DDE, and time to pregnancy. Time to pregnancy was censored at 13 months because women with longer times to pregnancy are more likely to have received treatment for infertility (41). Proportional hazards were verified for PCBs, DDE, and each covariate by using Schoenfeld residuals (42).

Fecundability odds ratios (43) were estimated by using a Cox proportional hazards model (44) modified for discrete time data, which is a proportional odds model (45) in SAS software (SAS version 9.0, SAS Institute, Inc., Cary, North Carolina). Fecundability odds ratios can be interpreted as the odds of achieving pregnancy for exposed women compared with the odds of achieving pregnancy for unexposed women for a given cycle, conditional on not having achieved a pregnancy in the preceding cycles. Therefore, fecundability odds ratios less than 1 suggest decreased fecundability (longer time to pregnancy), and fecundability odds ratios greater than 1 suggest increased fecundability (shorter time to pregnancy).

A priori, we decided that our base model of the association between PCBs, DDE, and time to pregnancy needed to adjust for triglycerides, cholesterol, and study center for methodological reasons, and for age (46) and smoking status (47, 48) (yes/no) because previous studies indicated an association with increased time to pregnancy. Covariates that changed the PCB/DDE and time-to-pregnancy beta coefficient by 10 percent or more were considered important confounders (49). We examined as confounders race (non-White/White), socioeconomic index, current or most recent occupation (never worked, blue collar, white collar), maternal prepregnancy body mass index, number of prior pregnancies (0, 1, 2 or more), infectious diseases (present/absent), chronic disease (present/absent), gynecologic condition (present/absent), irregular menstrual cycle (yes/no), previous fertility investigation (yes/no), history of sexually transmitted disease (yes/no), and gestational age at blood draw. We used −2 log-likelihood statistics comparing models with and without interaction terms for age, smoking status (yes/no), study center, and method of subject selection (random/nonrandom) to identify statistically significant effect modifiers at the 90 percent confidence level. We looked at the stratum-specific estimates to determine whether effect modification was important enough to report separate estimates of the association between PCB/DDE exposure and time to pregnancy. No covariates met these criteria. Our results reflect the fecundability of 390 women because, for 58 of the original 448 women, data were missing on time to pregnancy or covariates in the final model.

We assessed the shape of the dose-response curve by using categorization and splines. First, total PCB and DDE levels were categorized in the same groupings as those used in other analyses of these data (6, 10, 27, 50), and fecundability odds ratios and p values for continuous PCB and DDE levels (rescaled by their respective interquartile ranges) were used as trend tests. Regression splines with unknown knot locations were fit for PCBs and, separately in the same model, for DDE, adjusting for serum cholesterol, triglycerides, age, and smoking. We applied the Bayesian isotonic regression splines approach (51) by fitting a generalized additive model with random effects for woman and study center to improve efficiency and power. This approach incorporates a nonincreasing constraint on the regression functions while allowing the curves to be flat (corresponding to the null hypothesis of no association) with a prior probability of 0.5. We obtained posterior probabilities of a decreasing trend in fecundability with DDE and PCB level by updating this prior probability using information in the data. The overall weight of evidence in favor of a decreasing dose-response trend versus no association, assuming equal prior probabilities for the null and alternative hypotheses, was summarized by using Bayes’ factors (posterior odds of the alternative hypothesis) and posterior model probabilities. A Bayes’ factor greater than 1 suggests evidence in the data in favor of the alternative hypothesis. The posterior probability of no association was used as a Bayesian alternative to the p value. In addition, we estimated smoothed dose-response curves and 95 percent credible intervals (Bayesian alternative to confidence interval) by using the Bayesian isotonic regression splines approach.
We conducted several sensitivity analyses to check the robustness of our results against our representation of time to pregnancy (original; corrected for miscarriage; corrected for miscarriage, postpartum subfertility, and lactational amenorrhea) and the influence of subject selection (all subjects selected vs. those selected randomly). The majority of our study participants were White, and it took less time for Whites to become pregnant than non-Whites, so we restricted our analysis to Whites only to see whether the results were similar. We examined the 11 PCB congener-specific associations with time to pregnancy. First, each congener was examined in a separate model (11 separate models). Second, all PCB congeners were entered into the same model, where highly correlated (Pearson correlation coefficient greater than or equal to 80 percent) PCB congeners 105, 118, 138, 153, 170, 180, and 203 were added and entered as a single variable to avoid colinearity, and the remaining congeners were entered as individual variables.

**RESULTS**

Most women in our study were in their early twenties, White, and of mid- to upper socioeconomic status (table 1). Over 90 percent worked or had worked, over 60 percent had experienced at least one prior pregnancy, over 40 percent were current smokers, and less than 10 percent had a history of sexually transmitted infections or sterility investigation. Compared with women who became pregnant in 3 months or less, women who took more than 3 months to become pregnant were younger, more likely to smoke, and less likely to have experienced a prior pregnancy or to have had a history of sexually transmitted infections or sterility investigation. We found no significant associations between PCB levels and time to pregnancy, even after adjusting for potential confounders.

**TABLE 1. Characteristics of women in the Collaborative Perinatal Project, enrolled at 12 study centers in the United States from 1959 to 1965, who were trying to become pregnant**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All women (N = 390)</th>
<th>Time to pregnancy ≤3 months (n = 221)</th>
<th>Time to pregnancy &gt;3 months (n = 169)</th>
<th>Crude fecundability OR†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% or median</td>
<td>IQR‡</td>
<td>% or median</td>
<td>IQR</td>
</tr>
<tr>
<td>Median level of PCBs§ (µg/liter)</td>
<td>2.6 1.8, 3.6</td>
<td></td>
<td>2.5 1.8, 3.5</td>
<td></td>
</tr>
<tr>
<td>Median level of DDE¶ (µg/liter)</td>
<td>22.6 16.3, 32.8</td>
<td></td>
<td>22.1 15.6, 30.8</td>
<td></td>
</tr>
<tr>
<td>Median age (years)</td>
<td>23 20, 27</td>
<td></td>
<td>22 20, 25</td>
<td></td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>81.1</td>
<td></td>
<td>87.7</td>
<td></td>
</tr>
<tr>
<td>Non-White</td>
<td>18.9</td>
<td></td>
<td>12.3</td>
<td></td>
</tr>
<tr>
<td>Median socioeconomic index</td>
<td>5.7 4.0, 7.3</td>
<td></td>
<td>5.7 4.0, 8.3</td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>43.9</td>
<td></td>
<td>38.9</td>
<td></td>
</tr>
<tr>
<td>Median prepregnancy BMI</td>
<td>21.6 19.5, 24.2</td>
<td></td>
<td>21.3 19.4, 24.1</td>
<td></td>
</tr>
<tr>
<td>History of STDs</td>
<td>5.5</td>
<td></td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>No. of prior pregnancies</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>37.2</td>
<td></td>
<td>38.5</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>32.6</td>
<td></td>
<td>35.3</td>
<td></td>
</tr>
<tr>
<td>≥2</td>
<td>30.3</td>
<td></td>
<td>26.2</td>
<td></td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never worked</td>
<td>9.5</td>
<td></td>
<td>8.7</td>
<td></td>
</tr>
<tr>
<td>Blue collar</td>
<td>45.1</td>
<td></td>
<td>40.2</td>
<td></td>
</tr>
<tr>
<td>White collar</td>
<td>45.4</td>
<td></td>
<td>51.1</td>
<td></td>
</tr>
<tr>
<td>Irregular menstrual cycle</td>
<td>7.4</td>
<td></td>
<td>7.2</td>
<td></td>
</tr>
<tr>
<td>Gynecologic condition#</td>
<td>60.5</td>
<td></td>
<td>55.2</td>
<td></td>
</tr>
<tr>
<td>Sterility investigation</td>
<td>7.7</td>
<td></td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>Infectious disease</td>
<td>29.4</td>
<td></td>
<td>28.3</td>
<td></td>
</tr>
<tr>
<td>Chronic disease</td>
<td>9.8</td>
<td></td>
<td>9.6</td>
<td></td>
</tr>
</tbody>
</table>

* Some values were missing for prepregnancy body mass index (BMI (kg/m²)) (n = 35), history of sexually transmitted diseases (STDs) (n = 135), race (n = 3), occupation (n = 2), infectious disease (n = 2), and chronic disease (n = 2).
† Cox discrete time survival analysis was used to model the time to pregnancy, fecundability odds ratio (OR), and 95% confidence interval (CI) reported.
‡ IQR, interquartile range (quartile 1, quartile 3).
§ PCBs, polychlorinated biphenyls (per 1.795 µg/liter) rescaled by interquartile range.
¶ DDE, 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (per 16.6 µg/liter) rescaled by interquartile range.
# Includes vaginitis, incompetent cervix, surgery for incompetent cervix, leiomyoma, other gynecologic tumor, gynecologic surgery, other gynecologic reasons, and infertility.
pregnant tended to be older, non-White, employed in blue-collar occupations (recently or currently), of slightly lower socioeconomic status, current smokers, and more likely to have a history of sexually transmitted diseases, irregular menstrual cycles, gynecologic conditions such as vaginitis, and previous sterility investigation. These findings are similar to those from earlier studies (52–55). The Pearson correlation coefficient between serum PCB and DDE levels was 0.19.

Women in our study had planned their pregnancies. Demographically, a greater percentage of our study subjects (planners) were White, married, and of higher socioeconomic status compared with women in the Collaborative Perinatal Project whose pregnancies were unplanned (non-planners; data not shown). Planners were less likely than nonplanners to have a history of sexually transmitted diseases or to be regular contraceptive users, but nonplanners were less likely to have gynecologic conditions or a history of sterility investigation compared with planners. Compared with planners, nonplanners had a higher percentage of two or more prior pregnancies. Planners did not differ from nonplanners in terms of serum PCB or DDE levels during pregnancy, nor did they differ by age or menstrual cycle characteristics (data not shown).

The proportional hazards assumption was not violated for any of the variables. Forty-five pregnancies were censored at 13 months. The crude fecundability odds ratios were 0.89 (95 percent confidence interval (CI): 0.79, 1.00) for PCBs and 0.95 (95 percent CI: 0.84, 1.07) for DDE. Adjusting for the other organochlorine, triglycerides, cholesterol, study center, age, and smoking changed either fecundability odds ratio by less than 10 percent (adjusted fecundability odds ratio (OR) for PCBs = 0.92, 95 percent CI: 0.79, 1.07; adjusted fecundability OR for DDE = 1.00, 95 percent CI: 0.88, 1.15).

Time to pregnancy (table 2) increased for women in the highest exposure category for both PCBs (fecundability OR for PCBs ≥5.00 µg/liter = 0.65, 95 percent CI: 0.36, 1.18; p for trend = 0.27) and DDE (fecundability OR for DDE ≥60 µg/liter = 0.65, 95 percent CI: 0.32, 1.31; p for trend = 0.99) compared with women in the lowest exposure category (PCBs <1.24 µg/liter, DDE <14 µg/liter). When expressed on a lipid basis, increased time to pregnancy was attenuated slightly for women with higher serum PCB levels (fecundability OR for PCBs ≥600 ng/g = 0.89, 95 percent CI: 0.50 1.56; p = 0.68) and was no longer associated with higher serum DDE levels (fecundability OR for DDE ≥7,685 ng/g = 1.01, 95 percent CI: 0.52, 1.95; p = 0.98).

The estimated dose-response curves (figure 2) suggested that as serum PCB and DDE levels increased, the probability of conception decreased (time to pregnancy increased). The Bayes’ factors were 37.5 (posterior probability = 0.97) for PCBs and 4.8 (posterior probability = 0.80) for DDE by wet weight, indicating strong evidence for PCBs and suggestive evidence for DDE. Again, however, the evidence weakened considerably for PCBs assessed on a lipid basis (the Bayes’ factor decreased to 4.4). The evidence remained suggestive for DDE on a lipid basis (Bayes’ factor = 6.7). The observed associations between PCBs, DDE, and time to pregnancy were maintained regardless of how we represented time to pregnancy (original or corrected, see above) or whether we restricted the analysis to randomly selected pregnancies or to Whites only (data not shown). There were no PCB congener–specific associations with time to pregnancy, nor was there an association between the parent compound of DDE, DDT, and time to pregnancy (fecundability OR = 1.02, 95 percent CI: 0.93, 1.07; p for trend = 0.30).

DISCUSSION

Previous studies of PCB exposure and time to pregnancy have produced mixed results. In the New York State Angler Cohort, increased time to pregnancy was found for women exposed to PCBs when exposure was measured by using an index based on the total number of years eating Lake Ontario fish, portion size, number of species-specific fish meals, and species-specific PCB concentrations (17). In the same cohort, simpler exposure measures, based on fish consumption quantified by duration and frequency, were not related to time to pregnancy (16, 21). In the Swedish fishermen’s families cohort, overall, there was no evidence of a detrimental effect of PCB exposure on fertility, regardless of whether PCB exposure was measured by using a questionnaire (cohort affiliation, fish consumption, and growing up in a fishing village or fishing family) (18, 19) or back-calculated blood plasma or serum level (22, 23). However, when data on fishermen’s wives and sisters were combined, there was weak evidence of reduced time to pregnancy with increasing serum PCB levels (22, 23). In the Collaborative Perinatal Project, we found evidence suggesting an association between increasing serum PCB levels and increased time to pregnancy based on third-trimester serum organochlorine levels and time to pregnancy recalled at enrollment. Although our number of subjects with measured PCB levels was slightly larger than the Swedish fishermen’s families cohort, our results were still imprecise.

Our evidence for DDE exposure was less suggestive of an association with time to pregnancy. Our DDE results indirectly support recent evidence suggesting increased time to pregnancy with increasing DDT exposure (24); however, other recent evidence indicates a negative correlation with successful in vitro fertilization (4) and decreased time to pregnancy with increasing in utero exposure to DDE (36). These discrepancies may mean there is no true association between DDE exposure and time to pregnancy.

Generally, organochlorines measures on a wet-weight basis with adjustment for lipids are equivalent to lipid-basis measures. In the present study, the association between wet-weight PCBs and time to pregnancy attenuated when PCBs were expressed on a lipid basis (table 2, figure 2). Representing serum concentrations on a lipid basis, however, may introduce bias (57). Furthermore, the serum lipids conversion equation (33) used to estimate total lipids might not be appropriate for pregnant women (58). Therefore, we have greater confidence in the wet-weight results, where we adjusted for cholesterol and triglycerides separately (58).

Our maternal serum samples were analyzed for PCBs and DDE after being frozen for more than 30 years. PCB and DDE concentrations in breast milk samples from Swedish
### TABLE 2. Association between PCBs, DDE, and time to pregnancy based on 390 pregnancies† among women, enrolled in the Collaborative Perinatal Project at 12 study centers in the United States from 1959 to 1965, who were trying to become pregnant and for whom measured levels of serum organochlorine were available

<table>
<thead>
<tr>
<th>Organochlorine measure</th>
<th>No.</th>
<th>Fecundability OR*</th>
<th>95% CI*</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PCBs (wet weight)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCB level (μg/liter)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.00–1.24</td>
<td>48</td>
<td>Referent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.25–2.49</td>
<td>135</td>
<td>0.83</td>
<td>0.53, 1.29</td>
<td>0.40</td>
</tr>
<tr>
<td>2.50–3.74</td>
<td>120</td>
<td>0.88</td>
<td>0.54, 1.41</td>
<td>0.58</td>
</tr>
<tr>
<td>3.75–4.99</td>
<td>44</td>
<td>1.32</td>
<td>0.73, 2.39</td>
<td>0.36</td>
</tr>
<tr>
<td>≥5.00</td>
<td>43</td>
<td>0.65</td>
<td>0.36, 1.18</td>
<td>0.16</td>
</tr>
<tr>
<td>Total PCBs (per 1.795 μg/liter)</td>
<td>390</td>
<td>0.92</td>
<td>0.79, 1.07</td>
<td>0.27</td>
</tr>
<tr>
<td><strong>PCBs (lipid basis)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>PCB level (ng/g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–156</td>
<td>51</td>
<td>Referent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>157–290</td>
<td>130</td>
<td>1.01</td>
<td>0.65, 1.55</td>
<td>0.97</td>
</tr>
<tr>
<td>291–467</td>
<td>120</td>
<td>0.95</td>
<td>0.60, 1.48</td>
<td>0.80</td>
</tr>
<tr>
<td>468–599</td>
<td>44</td>
<td>1.30</td>
<td>0.74, 2.29</td>
<td>0.36</td>
</tr>
<tr>
<td>≥600</td>
<td>45</td>
<td>0.89</td>
<td>0.50, 1.56</td>
<td>0.68</td>
</tr>
<tr>
<td>Total PCBs (per 235 ng/g)</td>
<td>390</td>
<td>0.92</td>
<td>0.78, 1.09</td>
<td>0.34</td>
</tr>
<tr>
<td><strong>DDE (wet weight)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DDE level (μg/liter)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–14</td>
<td>74</td>
<td>Referent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15–29</td>
<td>202</td>
<td>0.96</td>
<td>0.67, 1.37</td>
<td>0.83</td>
</tr>
<tr>
<td>30–44</td>
<td>66</td>
<td>0.85</td>
<td>0.53, 1.35</td>
<td>0.48</td>
</tr>
<tr>
<td>45–59</td>
<td>29</td>
<td>1.15</td>
<td>0.64, 2.08</td>
<td>0.64</td>
</tr>
<tr>
<td>≥60</td>
<td>19</td>
<td>0.65</td>
<td>0.32, 1.31</td>
<td>0.23</td>
</tr>
<tr>
<td>Total DDE (per 16.6 μg/liter)</td>
<td>390</td>
<td>1.00</td>
<td>0.88, 1.15</td>
<td>0.99</td>
</tr>
<tr>
<td><strong>DDE (lipid basis)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DDE level (ng/g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–1,854</td>
<td>73</td>
<td>Referent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.855–3.665</td>
<td>202</td>
<td>0.93</td>
<td>0.65, 1.32</td>
<td>0.67</td>
</tr>
<tr>
<td>3.666–5.297</td>
<td>66</td>
<td>0.91</td>
<td>0.59, 1.42</td>
<td>0.69</td>
</tr>
<tr>
<td>5.298–7.684</td>
<td>29</td>
<td>1.15</td>
<td>0.63, 2.10</td>
<td>0.65</td>
</tr>
<tr>
<td>≥7.685</td>
<td>20</td>
<td>1.01</td>
<td>0.52, 1.95</td>
<td>0.98</td>
</tr>
<tr>
<td>Total DDE (per 1,965 ng/g)</td>
<td>390</td>
<td>1.03</td>
<td>0.91, 1.16</td>
<td>0.63</td>
</tr>
</tbody>
</table>

* PCBs, polychlorinated biphenyls; DDE, 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene; OR, odds ratio; CI, confidence interval.
† For 45 pregnancies, time-to-pregnancy estimates were longer than 13 months and were censored at 13 months.
‡ Fifty-eight of the original 448 pregnancies were excluded because of missing values: time to pregnancy (n = 21), PCBs (n = 12), and DDE (n = 25). For some women, multiple values were missing.
§ Adjusted for DDE, triglycerides, cholesterol, study center, age, and smoking. Total PCBs were rescaled by the interquartile range (1.795 μg/liter) to reflect an interpretable dose.
¶ Adjusted for DDE, triglycerides, cholesterol, study center, age, and smoking. Total PCBs were rescaled by the interquartile range (1.795 μg/liter) to reflect an interpretable dose.
# Adjusted for PCBs, triglycerides, cholesterol, study center, age, and smoking. Total PCBs were rescaled by the interquartile range (1.967 ng/g) to reflect an interpretable dose.
** Adjusted for PCBs, study center, age, and smoking. DDE was rescaled by the interquartile range (1.967 μg/liter) to reflect an interpretable dose.
mothers that were pooled and frozen at \(-20^\circ\text{C}\), then analyzed for PCBs and DDE 15 years and 25 years later, did not change over the 10-year period (59, 60). Therefore, our PCB and DDE estimates probably accurately reflected serum levels at the time of collection.

There are several potential limitations to our study. First, we had to focus on women who planned their pregnancy so we could obtain time-to-pregnancy estimates. Doing so excluded women who did not plan their pregnancy, which could be a problem if they had different PCB or DDE levels or differed regarding other important characteristics (61). When we compared planners and nonplanners, we did not find differences in PCB or DDE levels, nor did we observe differences in age or menstrual cycle characteristics.

Two other important groups of women were excluded from our study because of the design. First, we were not able to consider women who did not become pregnant, so the associations of PCB and DDE exposure with time to pregnancy could be stronger than those we observed. Second, we did not include women whose pregnancy ended in spontaneous abortion or stillbirth. Although no association was found between spontaneous abortion and PCBs in the New York State Angler Cohort (7) or the Swedish fishermen’s families cohort (18, 19), other studies have suggested an increased risk of spontaneous abortion with increasing PCB exposure (62, 63) and DDE exposure (9–11, 62). Therefore, we may have missed a group of women whose fecundability is sensitive to the effects of PCBs or DDE, so the associations could be stronger than what we observed.

Second, our results are dependent on the way in which PCBs and DDE were measured. We assumed that third-trimester maternal serum PCB and DDE levels accurately reflected prepregnancy serum PCB and DDE levels. Although levels could be different prior to pregnancy, evidence suggests that serum PCB and DDE levels are consistent over time in adult women (64, 65) and during pregnancy (28).

**FIGURE 2.** Probability of conception according to levels of serum polychlorinated biphenyls (PCBs; two left graphs) and 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE; two right graphs) measured in 390 pregnant women in the Collaborative Perinatal Project enrolled at 12 study centers in the United States from 1959 to 1965. Bayesian isotonic regression splines were applied to serum levels by wet weight (two upper graphs) and on a lipid basis (two lower graphs). Solid lines, posterior mean curves; dashed lines, pointwise 95% credible intervals.
Therefore, our third-trimester PCB and DDE levels should represent pre-pregnancy exposure reasonably well.

Third, our results are dependent on a crude measure of time to pregnancy. We used self-reported time to pregnancy, which has proven reasonably accurate (66, 67). However, there are different ways of interpreting the question, “How long did it take you to become pregnant?” with the response recorded in months, so some women may have been misclassified. We tried to reduce the amount of misclassification by correcting time-to-pregnancy estimates for miscarriage, postpartum subfertility, and lactational amenorrhea. Remaining misclassification should be nondifferential; although this misclassification may attenuate the association between time to pregnancy and PCB/DDE exposure, it is unlikely to explain the observed effects.

Fourth, not all women in our study were selected randomly. However, the observed associations were maintained when our analyses were restricted to women selected randomly, increasing our confidence that any bias was minimal.

Finally, we did not have information on several important risk factors including knowledge of the fertile period, frequency or timing of intercourse, stress, or occupational exposures (46), so we could not evaluate their effects on time to pregnancy. It is unlikely that any of these risk factors would have confounded the observed associations.

Experimental data provide little guidance on implicating a specific mechanism by which PCBs could increase time to pregnancy, although they do support the general biologic plausibility of effects. PCB 118 affects antithyroid (2) and antiestrogenic activity (68, 69) and also induces selected enzymes (68), and PCB 153 has been reported to be estrogenic (70) and induces several P-450 enzymes. Overall, however, the hormonal effects of PCB congeners present at the highest concentrations in our subjects are not well characterized (2). Furthermore, classification of these compounds according to hormonal activity in reviews of congener-specific effects, when available, is not reliable (68, 69).

Nonetheless, animals exposed to PCBs have experienced altered menstrual and estrous cycles, reduced numbers of follicles, amenorrhea, inhibited ovulation, and increased spontaneous abortions and resorption (1, 71, 72). In humans, Cooper et al. (6) found that high serum PCB levels were related to an estimated 1-day lengthening of the menstrual cycle among women in the Collaborative Perinatal Project. Menstrual cycle lengthening can result in longer time to pregnancy when time to pregnancy is measured in months, such as in our study. However, a 1-day increment would not have had an appreciable effect on time to pregnancy. Among other possibilities, high PCB exposure could increase the incidence of early loss (before a pregnancy is recognized) by adversely affecting either environmental conditions in the uterus necessary for maintaining a healthy pregnancy or development of the embryo (71).

A mechanism by which exposure to DDE could increase time to pregnancy is unclear. In human studies, DDE exposure has been associated with spontaneous abortion (9–11, 62) and reduced birth weight (73). In animal studies, DDE exposure adversely affected fertility and sexual development in male rats, probably because it blocks androgen binding with its receptor (3). Perhaps maternal DDE levels in our study serve as proxy paternal DDE exposure levels responsible for decreased fecundity, since time to pregnancy is a combined function of both male and female fertility (41).

In conclusion, evidence of an association between PCB or DDE exposure and time to pregnancy was inconclusive from these data but was suggestive for PCBs. PCBs and DDE were near peak production and use at the time that women were enrolled in the Collaborative Perinatal Project. Accordingly, exposure levels experienced by the women in our study were higher than those women experience in the United States today (74).

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Conflict of interest: none declared.

REFERENCES


