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# Polychlorinated biphenyl serum concentrations, lifestyle and time-to-pregnancy

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**BACKGROUND:** Consumption of fish contaminated with polychlorinated biphenyls (PCBs) and prenatal PCB serum concentrations have been associated with a longer time-to-pregnancy (TTP). However, the relationship between preconception serum PCBs concentrations and TTP has not been previously studied.

**METHODS:** Eighty-three women (contributing 442 menstrual cycles) planning pregnancies completed daily diaries regarding menstruation, intercourse, home pregnancy test results, and reported use of alcohol and cigarettes. TTP denoted the number of observed menstrual cycles required for pregnancy. Preconception blood specimens underwent toxicologic analysis for 76 PCB congeners via gas chromatography with electron capture; serum lipids were quantified with enzymatic methods. *A priori*, PCB congeners were summed into a total and three groupings—estrogenic, anti-estrogenic and other—and entered into discrete analogs of Cox models with time-varying covariates to estimate fecundability odds ratios (FOR) and corresponding 95% confidence intervals (CIs).

**RESULTS:** Estrogenic and anti-estrogenic PCB concentrations (ng/g serum) conferred reduced FORs in fully adjusted models (0.32; 95% CI 0.03, 3.90 and 0.01: 95% CI $<$ 0.00, 1.99, respectively). Reduced FORs (0.96) were observed for alcohol consumption standardized to a 28-day menstrual cycle in the same adjusted model (FOR = 0.96; 95% CI 0.93, 1.00).

**CONCLUSIONS:** These data suggest that environmental exposures including those amenable to change, such as alcohol consumption, may impact female fecundity. The findings are sensitive to model specification and PCB groupings, underscoring the need to further assess the impact of chemical mixtures on sensitive reproductive outcomes, such as TTP, especially in the context of lifestyle factors which are amenable to change, thereby improving reproductive health.

**Key words:** Keywordstime-to-pregnancy / polychlorinated biphenyls / fecundity / lifestyle / environment

Human conception is a non-random event determined in part by the couple's underlying fecundity or biologic capacity for reproduction in the context of environmental exposures and timing of sexual intercourse. The effect of hormonally active environmental chemicals on human reproduction has been the subject of considerable debate as recently reviewed (Torf *et al*., 2004; Buck Louis *et al*., 2006). Much of this early literature focused on dietary exposure, particularly the consumption of fish contaminated with persistent organochlorines, on subtle markers of human fecundity as measured by a longer time-to-pregnancy (TTP) (Axmon *et al*., 2000; Buck *et al*., 2000; Arakawa *et al*., 2003). Similarly, evidence suggestive of a trend ($P = 0.06$) for male lifetime sport-caught fish consumption and conception delay also has been reported (Courval *et al*., 1999). In a selected cohort of 390 pregnant women in their third trimester of pregnancy, a 35% reduction in fecundability, as measured by retrospectively reported TTP, was observed for women in the highest serum category of 11 polychlorinated biphenyls (PCB) congeners in comparison to women in the lowest category (Gesink Law *et al*., 2005). A study of 286 Swedish women asked to recall TTP for pregnancies years earlier reported higher fecundability ratios for women with serum PCB 153 concentrations $\geq$180 ng/g lipids in comparison to women with lower concentrations (Axmon *et al*., 2004). A cross-sectional study of 41 pregnant women and their partners reported a 78% reduction in fecundability associated with a blood mercury (an aquatic ecosystem contaminants) level $\geq$1.2 μg/l after adjusting for frequency of intercourse (Cole *et al*., 2006). While evidence to date is suggestive of a possible association between PCBs and TTP, past limitations include reliance on retrospectively reported TTP and women giving birth which, thereby, excludes couples unable to achieve pregnancy (Sallmen *et al*., 2000).

Recently, maternal serum PCB concentrations were estimated to decrease daily (−1.012 ng/g serum; $P < 0.0001$) in the interval from before conception to a HCG confirmed pregnancy (Bloom *et al*., 2007). If corroborated, these findings challenge the assumption that prenatal concentrations are valid estimates of preconception concentrations. While the reliability of retrospective TTP has been shown to be good, its validity as measured with a gold (prospective observation) standard has only been formally assessed in two studies. Validity was reported as good when follow-up was limited to 3–20 months (Zielhuis *et al*., 1992), but rather poor (17% exact agreement) when recall time was a decade (Cooney *et al*., in press). The absence of research focusing on the relation between preconception serum PCB concentration and TTP irrespective of women's ability to achieve pregnancy resulting in a live birth served as the impetus for study.

# Materials and Methods

## Design

A prospective cohort design was used to recruit women upon discontinuing contraception for the expressed purpose of becoming pregnant. The referent population comprised 2637 female participants aged 18–44 years in the New York State Angler Cohort Study who stated upon enrollment in 1991 that they were considering or undecided about future pregnancies (Vena *et al*., 1996). Among the 1031 women successfully located in 1996–1997, 787 (76%) were ineligible largely due to age or not desirous of pregnancy. Of the 244 women who met the study's eligibility criteria, 113 (46%) reported that they were planning to try to become pregnant within the next 6 months (the final eligibility criterion) and were interested in participating. Eleven participating women became pregnant prior to conducting the baseline interview and were excluded. An additional three women were found to be pregnant right after enrollment suggesting they also were pregnant at baseline and were excluded. Hence, 99 women enrolled in the study, with 83 women having completed diary cards for the calculation of TTP. Human subjects' approval was awarded for the study protocol and individual consent was obtained from all women prior to data collection.

## Data collection

*Baseline interview*

Every effort was made to enroll women at the time they were discontinuing contraception for purposes of becoming pregnant. Nurses interviewed women about their lifestyle and medical history then instructed them in the use of the Clearblue Easy™ home pregnancy test kits, reported to be capable of detecting $\leq$50 m IU of HCG. Women tested their first morning urine on the day menses was expected and one week later regardless of the first test result. Women were given 12 kits at baseline and, if needed, another 12 at 6 months to minimize stress associated with trying.

*Daily diaries*

The date of the last menstrual period reported at baseline was used to help women estimate when they might expect to begin daily data collection. Women were instructed how to complete daily diaries designed to capture menstruation, sexual intercourse, occasional use of birth control, cigarette smoking, alcohol and caffeine consumption, multivitamin usage and sport fish consumption. For count variables, women were asked to record daily the number of acts of vaginal–penile intercourse, number of cigarettes smoked, number of alcoholic beverages (beer, wine, wine coolers, hard liquor) and the number of caffeinated (coffee, tea, caffeinated soft drinks) beverages consumed. The diary comprised 52 preaddressed and postage-paid cards; reminder calls were made when diary cards were more than one week late. Data were censored on the day following participants' final study outcome: (i) the day following the first positive pregnancy test for women with live births; (ii) the day following a pregnancy loss or (iii) the day following 12 cycles with at least one act of intercourse during the fertile window in the absence of pregnancy.

*Biospecimen collection*

Approximately 25 cc of non-fasting blood was collected upon completion of the interview and represented the preconception specimen collection. Additional blood specimens were collected: (i) at the first prenatal visit or after a pregnancy loss regardless of gestational length; (ii) at 4–6 weeks postpartum; (iii) upon initiation and cessation of breastfeeding and (iv) after 12 unsuccessful months of attempting pregnancy with one act of intercourse during the estimated fertile window. The research nurse collected all blood specimens except the prenatal, which was collected as a part of the woman's clinical prenatal visit. Breast milk samples were collected from nursing mothers at approximately 4 weeks following delivery and upon weaning. Infants were prospectively followed for up to 24 months (Senn *et al*., 2005). Remuneration included $20 for each blood specimen and $10 for each breast milk sample.

## Toxicologic analysis

Blood specimens were processed using gas chromatography with electron capture detection (GC–ECD). Samples were run in batches of 10 including four additional quality control samples (i.e. one reagent blank, one serum blank, one quality control sample containing 15 calibration

standards at known values and one participant's duplicate sample) (Greizerstein *et al*., 1997; Bloom *et al*., 2007). Seventy-six PCB congeners were quantified including 64 single eluting and 12 di-eluting congeners and summed *a priori* into three groupings indicative of purported biologic activity (Cooke *et al*., 2001), plus a group of the total: (i) total PCBs, defined as the sum of 76 congeners; (ii) total estrogenic PCBs, defined as the sum of congeners #4_10, 5_8, 15_17, 18, 31, 44, 47, 48, 52, 70, 99, 101, 136, 153 and 188; (iii) anti-estrogenic PCBs, defined as the sum of congeners #77_110, 105, 114, 126, 171_156 and 169 and (iv) all remaining PCBs. PCB concentrations were corrected only for recovery; all observed values were used in the analysis to minimize potential bias introduced by substituting values below the limits of detection (Schisterman *et al*., 2006). Total serum lipids (TL) were quantified using enzymatic methods as the function of total cholesterol (TC), free cholesterol (FC), triglycerides (TG) and phospholipids (PL) using the following formula: $TL = 1.677\ (TC - FC) + FC + TG + PL$ (Phillips *et al*., 1989). Serum PCBs were expressed as ng/g serum (equivalent to parts per billion) to avoid potential biases associated with lipid standardization (Schisterman *et al*., 2005); serum lipids were expressed in mg/dl.

## Operational definitions

*Menstrual cycle and TTP*

The daily diary asked women to report any bleeding or spotting regardless of whether it was time for their period using the following rating: 0, none; 1, spotting; 2, light; 3, moderate and 4, heavy. We defined menstruation as any report of spotting or bleeding that was followed within 1 day by at least two additional days of bleeding or spotting regardless of intensity. A menstrual cycle was defined as the interval (in days) between the first day of menstrual bleeding to the next first day of menstrual bleeding as reported on the diary. TTP was defined as the number of menstrual cycles required for pregnancy as measured by a positive pregnancy test. Women who immediately became pregnant before the first prospectively captured menses are defined as having becoming pregnant in cycle zero. These women were enrolled mid-cycle and typically contributed 1–2 weeks of data. Given the absence of a biomarker of ovulation for the study, we used the Ogino–Knaus method for estimating the likely date of ovulation by counting back 14 days from the end of the cycle (Knaus, 1929; Ogino, 1930). This approach is in keeping with greater cycle variability in the secretory rather than proliferative phase of the menstrual cycle (Saito *et al*., 1972). The fertile window was purposefully broadly defined as an 8-day window commencing 5 days before the presumed date of ovulation and ending 2 days after ovulation.

*Fecundity covariates*

Parity was defined as the number of live births reported at the baseline interview. Lifestyle was defined as the number of cigarettes smoked or alcoholic beverages (including beer, wine, wine coolers and hard liquor) reported on daily diaries and were summed and standardized to a 28-day menstrual cycle to allow per cycle comparison while controlling for women's varying menstrual cycle lengths. This was done by taking the reported per cycle consumption, multiplying by 28 and then dividing by the number of days in the cycle. Failure to standardize would have resulted in women with longer cycles looking like they consumed more, as they had more opportunity for consumption on a per cycle basis. Women were asked whether they had vaginal–penile intercourse each day, though frequency for our analysis was restricted to the number of days with at least one intercourse during the fertile window.

## Statistical analysis

The descriptive phase of analysis included assessing the distributions of PCBs and TTP and the comparison of women's baseline characteristics by pregnancy status to identify factors influential for pregnancy. Separate PCB models were developed to delineate congener groupings that might be informative for female fecundity using menstrual cycles as the unit of analysis. The first model included serum PCB concentration as a simple sum of all PCB congeners, while the second model *a priori* categorized PCB congeners by purported biologic activity, i.e. estrogenic, anti-estrogenic and other (Cooke *et al*., 2001). Frequency of sexual intercourses during the estimated fertile window served as a covariate in every model to minimize potential confounding (Stanford and Dunson, 2007). Two other types of covariates were considered in the analysis: those constant across time (serum PCB and lipid concentrations, age, parity) and those that are varying with each cycle (standardized amount of cigarette and alcohol consumption). To accommodate the time-varying frequency of intercourse, a discrete analog of the Cox model with time-varying covariates was used as the generic model for all of the TTP analyses. Natural logarithms of the odds of becoming pregnant were modeled as a linear function of covariates for each cycle, and the results were combined by cycle. The fecundity odds ratio (FOR) denotes the odds of becoming pregnant among women with higher PCB concentrations in comparison to women with lower PCB concentrations, given all other covariates being equal. A FOR $<1$ denotes reduced fecundability or a longer TTP, whereas an FOR $>1$ denotes increased fecundability or a shorter TTP. All women who contributed at least one cycle (including cycle zero) were considered in the analysis. For a given cycle number, all women who did not become pregnant before the given cycle and who were not lost to follow-up were considered to be at risk for becoming pregnant.

For models with PCB exposure and frequency of intercourse, the generic model described above was used. Two women were missing parity, while 30% of women had one or more individual lipid components missing necessitating the use of multiple imputation techniques for estimating that missing fraction of total serum lipids (Phillips *et al*., 1989). This arose because insufficient serum remained for lipid analysis following the quantification of PCBs. The pattern of missing data was used to calculate lipids. In the first stage, the Markov Chain Monte Carlo method was used to create five sets with imputed values of TC, FC, TG and PL to obtain the monotonic structure of missing values. Natural logarithms of these variables were used, given their skewed distributions, along with age, estrogenic, anti-estrogenic and other PCB groupings, and average alcohol and cigarette consumption in a standardized menstrual cycle. After obtaining monotonic patterns of missing data, propensity scores were used to impute the remaining values of TC, FC, TG and PL. In the last stage, logistic regression was used to impute values of missing parities for two women. For each imputed set, lipid values were calculated (Phillips *et al*., 1989). The results for the generic models applied to each imputed data set were combined (SAS procedure MIANALYZE) to obtain estimates, SEs and confidence interval (CI) adjusted for imputed sets. As a part of sensitivity analysis, generic models using PCB tertiles (in lieu of continuous) were fitted. A value of $P < 0.05$ was considered significant.

# Results

Among the 99 women who enrolled and completed a baseline interview, 83 (84%) women (contributing 442 menstrual cycles) were available for analysis after excluding women who failed to return any diary cards or with insufficient blood volume for toxicologic analysis. Menstrual cycles were the unit of analysis. Excluded women were not systematically different from those who were included with regard to TTP or pregnancy status (data not shown).

The distribution of the number of cycles contributed by women included: 12 women contributing $<1$ cycle (conceived in cycle 0); 16

**Table I Description of cohort at enrollment by pregnancy status ($n = 83$)**

| Characteristic | Pregnant ($n = 62$), $n$ (%) | Not pregnant ($n = 21$), $n$ (%) |
|---|---|---|
| Age (in years): | | |
| 20–29 | 25 (40) | 8 (38) |
| 30–34 | 37 (60) | 13 (62) |
| Mean (±SD) | 30.1 (±2.37) | 30.0 (±2.73) |
| Education | | |
| High school | 6 (10) | 3 (14) |
| College | 46 (74) | 16 (76) |
| Graduate school | 10 (16) | 2 (10) |
| Gravidity | | |
| Nulligravida | 13 (21) | 7 (33) |
| Multigravida | 49 (79) | 14 (67) |
| Parity | | |
| Nulliparous | 15 (24) | 8 (38) |
| Multiparous | 47 (76) | 13 (62) |
| Average number of acts of unprotected intercourse per menstrual cycle[a] | 2.19 (±1.42) | 2.26 (±1.10) |
| Reported smoking while trying to conceive | 14 (22) | 6 (29) |
| Average number or cigarettes smoked per menstrual cycle[a,b] | 185.31 (±173.34) | 21.90 (±43.21) |
| Percentage who reported drinking alcoholic beverages while trying to conceive[a] | 49 (79) | 19 (90) |
| Average number of alcoholic beverages consumed per menstrual cycle[a,c] | 9.18 (±10.81) | 15.45 (±14.59) |

Not pregnant includes 10 women who did not become pregnant and 11 women who withdrew from the study without a prospectively observed pregnancy.
[a]Standardized to a 28-day menstrual cycle inclusive of the fertile window; [b]$P < 0.05$ (two-sided); [c]$P = 0.05$.

**Table II Polychlorinated biphenyl (PCB) congener tertiles by pregnancy status ($n = 83$)**

| Tertiles of PCBs (ng/g serum) | Pregnant ($n = 62$), $n$ (%) | Not pregnant ($n = 21$), $n$ (%) |
|---|---|---|
| Total PCBs | | |
| First (10.65–14.53) | 17 (27) | 10 (48) |
| Second (14.53–16.16) | 21 (34) | 6 (29) |
| Third (16.16–32.76) | 24 (39) | 5 (24) |
| Estrogenic PCBs | | |
| First (1.52–2.07) | 17 (27) | 9 (43) |
| Second (2.07–2.28) | 22 (35) | 7 (33) |
| Third (2.28–4.53) | 23 (37) | 5 (24) |
| Anti-estrogenic PCBs | | |
| First (0.03–0.16) | 20 (32) | 7 (33) |
| Second (0.16–0.22) | 20 (32) | 7 (33) |
| Third (0.22–0.65) | 22 (35) | 7 (33) |
| Other PCBs | | |
| First (11.46–12.24) | 17 (27) | 10 (48) |
| Second (12.24–13.71) | 21 (34) | 7 (33) |
| Third (13.71–29.15) | 24 (39) | 4 (19) |

Not pregnant includes 10 women who did not become pregnant and 11 women who withdrew from the study without a prospectively observed pregnancy. None of above differences were statistically significant.

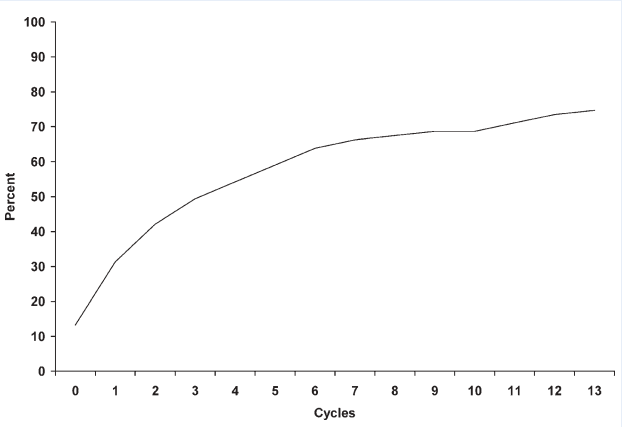


**Figure 1** Cumulative pregnancy distribution, New York State Angler Prospective Pregnancy Study.

contributing 1; 11 contributing 2; 7 contributing 3; 5 contributing 4; 5 contributing 5; 6 contributing 6; 3 contributing 7; 1 contributing 8; 1 contributing 9; 2 contributing 11; 8 contributing 12 and 6 contributing ≥13 cycles. Among the 83 women participating women, 48 had a live birth, 14 experienced pregnancy losses and 10 did not become pregnant within 12 at-risk menstrual cycles. Eleven women were lost to follow-up with a mean drop out time of 4.7 (±4.4) cycles.

Few significant differences were observed with regard to factors believed relevant for pregnancy with the exception of cigarette smoking and alcohol consumption (Table I). Women not achieving pregnancy reported consuming 15.45 (±14.59) alcoholic beverages per standardized menstrual cycle in comparison to 9.18 (±10.81) beverages consumed by women achieving pregnancy ($P = 0.05$). Conversely, cigarette usage per cycle was significantly ($P < 0.05$) higher among women who did versus did not become pregnant (i.e. 185.31 and 21.90, respectively). No significant differences in PCB concentrations (in tertiles) were observed by women's ability to become pregnant or not (Table II).

Enrolled women were followed for up to 12 at-risk cycles or those with at least one act of intercourse during the estimated fertile window, resulting in lengths of participation ranging from 0 to 21 cycles. Fig. 1 presents the cumulative pregnancy distribution among study participants beginning with cycle zero. Among the 83 women, 41 (49.4%) were pregnant by cycle three, with 53 (63.9%) and 61 (73.5%) by cycles 6 and 12, respectively.

Fig. 2 presents the overall conditional TTP distribution by cycle (Fig. 2a) and then stratified by tertile of total PCB concentration (Fig. 2b). The highest probability of pregnancy was observed in cycle one (21.1%) with 13.3 and 16.4% in cycles zero and two, respectively (Fig. 2a). It should also be noted that pregnancy probabilities remained relatively high even after six cycles, with cycles 11 and 12 having probabilities of 12.5 and 14.3%, respectively. TTP distributions varied by PCB tertile (Fig. 2b). Specifically, cycle six conferred the highest probability for the lowest PCB tertile and cycles one and two for the middle and highest tertiles, respectively. Of added note is the occurrence of pregnancy through cycle 13 for women in the upper two tertiles, whereas all pregnancies occurred by cycle nine for women in the lowest exposure tertile.

Table III reflects strong correlations ($r \geq 0.88$; $P < 0.0001$) between total, estrogenic and other PCB groupings. Conversely, anti-estrogenic PCB congeners were not correlated with estrogenic PCBs ($r = 0.10$; $P = 0.40$).

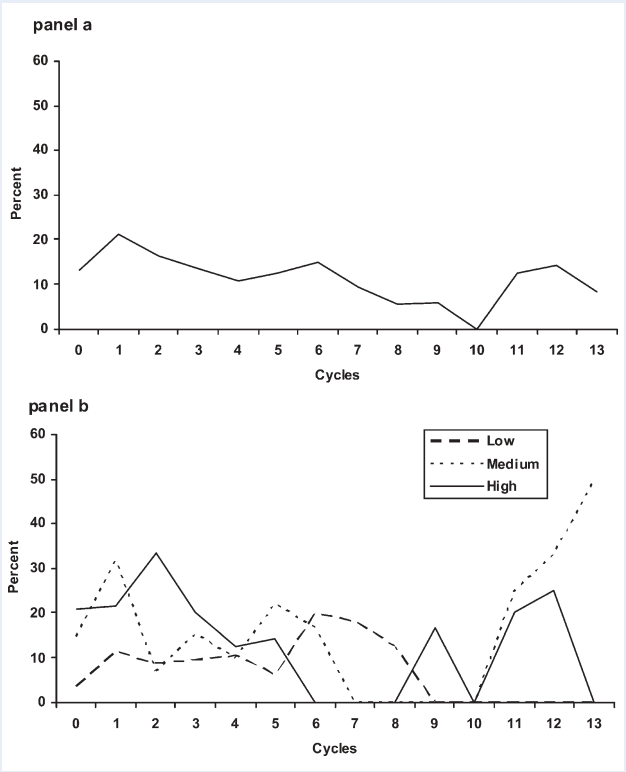


**Figure 2** Conditional time to pregnancy distributions for overall cohort (**a**) and by tertile of total polychlorinated biphenyl concentration (**b**), New York State Angler Cohort Prospective Pregnancy Study.

As shown in Table IV, total serum PCBs were observed to increase the FOR indicative of a shorter TTP after adjusting for frequency of intercourse during the fertile window (FOR = 1.08; 95% CI 1.00, 1.16) or all covariates (i.e. serum lipids, age, parity and usage of cigarettes and alcohol) (FOR = 1.10; 95% CI 1.00, 1.20). In addition, alcohol consumption significantly reduced the FOR in the fully adjusted model (FOR = 0.96; 95% CI 0.93, 0.99). Estimated FORs revealed an interesting pattern when PCB congeners were grouped by purported biologic activity. Reductions in FORs, denoting a longer TTP, were observed for estrogenic and anti-estrogenic PCBs, while all other PCB congeners conferred an increased FOR denoting a shorter TTP. None of these differences were statistically significant. Of added note is the absence of any relation between serum lipids and TTP in any of the adjusted models. In all of the models, frequency of intercourse significantly increased the FOR by ~20%. Alcohol consumption was associated with a longer TTP in both models, though in model 4 the CI included one when rounding the upper bound to two-decimal places.

## Discussion

When summed as a simple total, PCB concentrations were associated with a FOR hovering around 1.0 denoting the absence of an effect. Estrogenic and anti-estrogenic PCB groupings were associated with a reduced FOR, suggesting the importance of assessing exposure in relation to biologic classifications rather than a simple sum of individual congeners. The absence of significant correlations between estrogenic and anti-estrogenic groupings may provide some evidence that these groupings are exerting an independent effect in relation to TTP. However, the absence of a standardized approach for grouping PCB congeners with regard to biologic activity precludes full interpretation of the findings (Hansen, 1998).

Previous authors have assessed select PCB congeners in relation to TTP among women whose pregnancies resulted in live births, and reported conflicting evidence with regard to fecundity (Axmon *et al*., 2004; Gesink Law *et al*., 2005). Gesink Law *et al*. (2005) looked at select PCB congeners based upon serum samples obtained during mid-pregnancy and retrospectively reported TTP, while Axmon *et al*. (2004) collected bloods years following delivery and assessed only PCB #153. Subsequently, Axmon and colleagues assessed serum PCB #153 and 1,1-dichloro-2,2-bis (*p*-chlorophenyl)-ethylene (*p,p′*-DDE) concentrations in relation to retrospective TTP for four distinct geographical samples of pregnant women. An adverse relation

**Table III Correlations for PCB groupings**

| PCB grouping | Total PCBs | Estrogenic PCBs | Anti-estrogenic PCBs | Other PCBs |
|---|---|---|---|---|
| Total PCBs | 1.00 | 0.91* | 0.43* | 1.00* |
| Estrogenic PCBs | 0.91* | 1.00 | 0.10 | 0.88* |
| Anti-estrogenic PCBs | 0.43* | 0.10 | 1.00 | 0.45* |
| Other PCBs | 1.00* | 0.88* | 0.45* | 1.00 |

Pearson correlation coefficients (rounded to two-decimal places) based upon observations for 83 women. *$P < 0.0001$.

**Table IV Serum PCB concentrations and adjusted fecundability odds**

| PCB grouping model | FOR | 95% CI |
|---|---|---|
| Model 1 ($n = 83$; 447 cycles) | | |
| Σ PCBs | 1.08 | 1.00, 1.16 |
| Frequency intercourse[a] | 1.17 | 1.00, 1.38 |
| Model 2 ($n = 81$; 444 cycles) | | |
| Σ PCBs ($P = 0.041$) | 1.10 | 1.00, 1.20[b] |
| Serum lipids | 1.00 | 1.00, 1.00 |
| Frequency intercourse[a] | 1.21 | 1.03, 1.43 |
| Age | 1.02 | 0.89, 1.17 |
| Parity | 1.35 | 0.85, 2.13 |
| Cigarette usage | 1.00 | 1.00, 1.00 |
| Alcohol usage ($P = 0.022$) | 0.96 | 0.93, 0.99 |
| Model 3 ($n = 83$; 447 cycles) | | |
| Estrogenic PCBs | 0.42 | 0.06, 3.11 |
| Anti-estrogenic PCBs | 0.02 | <0.00, 3.59 |
| Other PCBs | 1.33 | 0.90, 1.97 |
| Frequency intercourse[a] | 1.19 | 1.01, 1.39 |
| Model 4 ($n = 81$; 444 cycles) | | |
| Estrogenic PCBs | 0.32 | 0.03, 3.89 |
| Anti-estrogenic PCBs | 0.01 | <0.00, 1.99 |
| Other PCBs | 1.44 | 0.88, 2.37 |
| Serum lipids | 1.00 | 1.00, 1.00 |
| Frequency intercourse[a] | 1.23 | 1.04, 1.45 |
| Age | 1.03 | 0.90, 1.18 |
| Parity | 1.46 | 0.91, 2.33 |
| Cigarette usage | 1.00 | 1.00, 1.00 |
| Alcohol usage ($P = 0.025$) | 0.96 | 0.93, 1.00[b] |

Serum PCBs were expressed as ng/g serum and serum lipids as mg/dl, age and parity left continuous, and cigarette and alcohol use standardized to woman's average menstrual cycle. [a]Restricted to number of days with sexual intercourse during the fertile window; [b]CIs exclude 1.0 before rounding to two decimal places. FOR, fecundability odds ratio; CI, confidence interval.

was only observed for the Greenlandic sample (Axmon *et al*., 2006a). We looked only at PCB #153 and observed an elevated FOR that fluctuated between 1.32 and 10.36 depending upon the covariates in the model; all CIs included one. Combined, these findings underscore the importance of assessing all congeners possible, rather than relying on a single congener, to minimize the probability of an erroneous conclusion. In addition, comparisons require caution given the potential for regional differences in human congener profiles and the many possible differences with regard to laboratory practices, viz., quantification capabilities, automatic substitution of values below the limit of detection or lipid adjustment.

Of added importance is the relation between frequency of intercourse and female fecundability. Regardless of model, every additional day with at least one act of intercourse during the estimated fertile window conferred on average a 20% reduction in TTP, though considerable couple heterogeneity is likely limiting our ability to quantify the reduction, *per se*. An important limitation underlying this finding is the absence of a biomarker of ovulation to help refine our fertile window, which has been suggested to comprise the 5 days before and the day of ovulation (Wilcox *et al*., 2000; Fehring *et al*., 2006). However, a range of <1 to >5 days was found in a recent study of subfertile couples (Keulers *et al*., 2007). Our fertile window relies heavily upon ovulation occurring ~14 days before the onset of the next expected menses, and assumes women's ability to accurately record bleeding. In addition, our models assume that acts of intercourse are independent despite limited empirical evaluation of this assumption.

Another important finding is the 4% reduction in the FOR for each alcoholic beverage consumed within a standardized 28-day menstrual cycle. Of added note is the tight CI for this estimate and its consistency across models. Even when stratifying our analysis by parity in the event it is an intermediate variable, the FORs were below one with CIs included of one. Our finding for alcohol may support the need for prospective measurement and women's willingness to report in light of data suggestive of an effect. To our knowledge, this is the first study to report a reduction in female fecundability based upon preconception enrollment and prospective longitudinal (daily) capture of alcohol consumption and HCG confirmed pregnancies. A previous prospective pregnancy study reported a significant reduction in fecundability with alcohol consumption, which was ascertained on a monthly basis while attempting pregnancy (Jensen *et al*., 1998). Other studies reported no associations when relying on couples' retrospective reporting of alcohol consumption and/or TTP (Curtis *et al*., 1997; Olsen *et al*., 1997; Hassan and Killick, 2004). Subsequently, women consuming wine were reported to have a reduced probability of a TTP >12 months suggesting the possible importance of assessing type of alcoholic beverage (Juhl *et al*., 2003). Our finding requires careful attention in that we did not assess specific types or quantity of alcohol consumption, though we found no evidence that women's alcohol consumption changed over the course of trying to conceive (data not shown).

While limited in terms of cohort size, this study has several important strengths including the preconception enrollment of women and an ability to separate cycle zero from cycle one for estimating FORs in relation to PCB exposure and other covariates believed relevant for female fecundity. To this end, women pregnant in cycle one had one full cycle observed in the study, whereas women pregnant in cycle zero had less than one full cycle observed. Prospective capture of pregnancy was based upon a sensitive home pregnancy test kit, which was suitable for storage at room temperature, unaffected by many common pharmaceuticals and not dependent upon first morning void. We further assessed the validity and reliability of the pregnancy test by asking the first 20 enrolled women to use and record the results from two different home pregnancy tests. We observed complete agreement for test results. Another important study strength is the selection of women from a specified 16 county geographic area to minimize geographical variations in fecundity across larger regions (Juul *et al*., 1999).

Cautious interpretation is further required in that PCB quantification was based on GC–ECD. We recognize the potential for false-positive results, given the lower specificity and selectivity of the GC–ECD approach in comparison to mass spectrometry (Garrido *et al*., 2000), and the relatively low concentrations, near laboratory detection limits, for some congeners.

Despite the couple-dependent nature of human fecundity, our cohort was limited to women. We had no information on male partners' exposures, including alcohol consumption, despite experimental

evidence suggesting that male rats fed alcohol during the peripubertal period sired pregnancies with smaller litters and less likely to be carried to term in comparison to unexposed animals (Emanuele *et al*., 2001). The authors interpreted these findings as being suggestive of testicular oxidative injury and, possibly, germ cell apoptosis.

The need for couple-based preconception designs has been previously articulated (Buck *et al*., 2004). Despite the identification of environmental exposures that may impact fecundability as measured by TTP, most have relied upon retrospective reporting of TTP or baseline covariate data rather than prospective longitudinal capture of both. This methodologic deficiency may account for our inability to explain the heterogeneity in couple fecundity. For example, Axmon and colleagues reported that only 14% of the variance in TTP was explained by oral contraceptive use, menstrual cycle length, age at conception and parity (Axmon *et al*., 2006b). Moreover, our limited understanding of the relation between environment and human fecundity precludes a more complete interpretation of the effect of chemical exposure in the context of lifestyle factors and particularly frequency of sexual intercourse, a necessary but not sufficient criterion for pregnancy. Suffice it to say, continued reliance on a single or select grouping of PCB congeners may obfuscate rather than delineate their effect on sensitive markers of human reproduction, such as TTP. Our findings underscore the importance of assessing chemical exposure in the context of lifestyle factors (sexual intercourse and alcohol consumption) that may exert effects on fecundity, particularly given that they may be modifiable.

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