Environmental Organochlorines and Semen Quality: Results of a Pilot Study

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There have been numerous studies that suggest that sperm concentrations (sperm counts) are declining in men. However, other studies suggest that sperm counts are not declining or may be increasing in some areas. Although there is disagreement on whether there is a downward temporal trend in sperm counts, the studies provide evidence that sperm counts vary by geographic location. It has been hypothesized that the geographic variation in sperm concentrations may be due to environmental exposures, lifestyle factors, or some unknown causes. To determine whether contemporary ambient levels of polychlorinated biphenyls (PCBs) and p,p′-DDE are associated with altered semen quantity and quality, we selected a study population without specific exposure to PCBs or p,p′-DDE. The present study presents the results from a pilot study on the relationship between serum PCBs and p,p′-DDE and semen quality in 29 subjects recruited from the Massachusetts General Hospital Andrology Laboratory. Of the 29 subjects, 3 had sperm concentrations < 20 million/mL, 7 had < 50% motile sperm, 9 had < 4% normal morphology, and 6 were below normal in one semen parameter. The 18 subjects with normal spermatozoa concentration, motility, and morphology were used as comparison subjects. The mean (SE) concentration of the sum of PCBs and p,p′-DDE was 242 ng/g lipids (34.0) and 354 ng/g lipids (120), respectively, for men with below normal motility as compared to 202 ng/g lipids (16.6) and 240 ng/g lipids (31.1), respectively, for the comparison subjects. The data showed general trends that were suggestive of an association between PCBs and p,p′-DDE and abnormal motility, as well as with sperm concentration and morphology. A full-scale study is currently in progress.

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Currently there is scientific and public concern about whether exposure to ambient levels of so-called endocrine disruptors, such as p,p′-DDE [1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene], a metabolite of DDT [1,1,1-trichloro-2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane], and polychlorinated biphenyls (PCBs), alter semen quality (1,2). This concern stems from studies showing that PCBs and p,p′-DDE are found in a large proportion of the general population (3–5), as well as animal and human studies suggesting possible associations of exposure to PCBs and p,p′-DDE with semen abnormalities and altered testis function (6–8). The U.S. Environmental Protection Agency (EPA) National Human Adipose Tissue Survey estimated that > 95% of the U.S. population had detectable levels of PCBs (9). It is estimated that > 99% of individuals have detectable blood levels of p,p′-DDE (4).

PCBs and p,p′-DDE, the most stable metabolite of DDT, are persistent, lipophilic chemicals that are suspected endocrine disruptors. DDT was widely used as an insecticide, whereas PCBs were used in cutting oils, lubricants, and as electrical insulators. Although their use and manufacture were banned nearly 30 years ago, they are ubiquitous and persist in the environment; PCBs are distributed worldwide as environmental pollutants and have been measured in air, water, aquatic, and marine sediments, and in fish and wildlife (10). Furthermore, they are biologically concentrated and stored in human adipose tissue. The general population continues to be exposed to PCBs and p,p′-DDE through ingestion of contaminated food (fish, meat, milk, and their by-products) and water, dermal contact (soil and house dust), and inhalation (indoor air in buildings that have various sources).

Many studies have suggested that sperm concentrations (sperm counts) are declining in men (2, 11–15). However, other studies suggest that sperm counts are not declining or may have increased marginally in some areas (16–19). Although there is disagreement on whether there is a downward temporal trend in sperm counts, the studies provide evidence that sperm counts vary by geographic location (20). It has been hypothesized that the geographic variation in sperm concentrations may be due to environmental exposures, lifestyle factors, or some unknown causes (20). However, the population studies published to date lack information at the individual level on lifestyle factors, such as cigarette smoking, which may adversely affect semen quality, as well as information on other potentially important environmental exposures (21,22). Such studies were not designed to investigate the relationship between environmental exposures and sperm concentration. Therefore, they are unable to test the hypothesis that environmental agents are associated with altered sperm counts.

To determine whether contemporary ambient levels of PCBs and p,p′-DDE are associated with altered semen quantity and quality, we selected a study population without specific exposure to PCBs or p,p′-DDE. Detecting even an association of small magnitude may have large population effects because of the widespread distribution of PCBs and p,p′-DDE in the general population.

Materials and Methods

The study was explained by the research nurse to each potential subject, and any questions they had were answered before the subjects signed the consent form approved by the Harvard School of Public Health Institutional Review Board and the Massachusetts General Hospital (MGH) Human Subjects Committee.

Men presenting to the MGH Andrology Laboratory for semen evaluation were asked to participate. The number of men recruited was limited because this study was funded as a pilot study. Most of these men were partners in couples undergoing medical evaluation for an inability to conceive a pregnancy. An individual man may or may not be infertile/subfertile because the couple’s fertility
depends on both male and female fertility. This population was chosen for study for three reasons. First, we wanted to study a population without specific (i.e., occupational) exposure to PCBs or \( p,p' \)-DDE. The exposure of these men therefore represents contemporary ambient levels. Second, the andrology laboratory population represents men who are motivated to participate in the study, making the study both feasible and less subject to potential selection bias. Third, the andrology laboratory population is diverse, providing a population of men who are representative of men in other clinics.

Semen analysis. Semen collection and analyses were performed at MGH in a clinical setting and standardized manner. Because the patient was already providing a semen sample for clinical evaluation, we asked the patient if the results of this sample could be used for the research study. The andrologist analyzed the semen sample without knowledge of the condition; the serum samples were analyzed for PCBs and \( p,p' \)-DDE several months after the patient’s visit. All patients are instructed by the clinic (routine previsit instructions) to refrain from sexual activity for at least 3 days before providing the semen sample at the clinic, and information on the period of abstinence (in days) was collected.

Semen was collected in a sterile container and allowed to liquefy for 20 min. The physical properties of the semen, including the sample volume, pH, color, and viscosity were recorded. We measured spermatozoa counts and motility by computer-assisted semen analysis (CASA) using the Hamilton Thorne IVOS 10 Analyzer (Hamilton-Thorne Research, Beverly, MA). To minimize variability (28), we used a constant analysis setup and performed additional quality control steps including playback and viewing of quality control plots in subjects with counts below 20 million/mL and above 50 million/mL. To assess sperm morphology, a seminal smear was made on a glass slide with < 5 µL of semen, stained with Diff-Quik. We assessed 200 sperm using the Tygerberg Strict Criteria (24). We used benzidine hydroxide staining (25) to detect the presence of leukocytes in the semen and distinguish them from other round cells, including immature germ cells.

We used the World Health Organization (WHO) reference values for evaluation of infertility to define below normal values for sperm concentration (< 20 million spermatozoa/mL) and sperm motility (< 50% motile sperm or < 10 million total motile spermatozoa, accounting for individuals with low total sperm counts but high percent motility) (26). The WHO reference values are based on manual counting and manual assessment of motility. The MGH study reference values for sperm concentration and sperm motility are similar to those recommended by the WHO (26). The morphology criteria used to determine below normal morphology was < 4% normal morphology (Tygerberg Strict Criteria for morphology) (26). Men that were above normal in all three semen parameters (sperm count, motility, and morphology) were considered to have normal semen and were used as a comparison population.

Questionnaire. All subjects were asked to complete a self-administered detailed medical history and lifestyle questionnaire. Each subject was given the questionnaire after he produced the semen sample and was asked to complete the questionnaire at home and return it by mail within 1 week. At the time of the clinic visit for the semen analysis, the subject was asked to report abstinence time and medication use during the last 3 months.

Semen PCB, \( p,p' \)-DDE, HCB measurements. Blood serum samples were analyzed by the organic chemistry analytical laboratory at the Harvard School of Public Health. Target analytes included 65 individual PCB congeners, \( p,p' \)-DDE, and hexachlorobenzene (HCB). Details of the sampling, analytical, and quality control procedures are described elsewhere (27). Briefly, the blood samples were collected in red top vacutainer tubes, and the serum fraction was separated by centrifugation. Samples were stored in solvent-rinsed glass vials with Teflon-lined caps at –80°C until analysis.

For the extraction, we used procedures developed by the Centers for Disease Control (28) with modifications to conform to ultra-trace–level analyses. These modifications included additional cleaning of glassware and dry reagents used in the column chromatography cleanup and reducing the final extract volume to 100 µL.

We measured percent lipid for each serum sample gravimetrically by weighing an aliquot of sample extract evaporated to dryness. The mean ± SD percent lipid for the 29 samples were 0.75% ± 0.19. The serum extracts were analyzed by gas chromatography with electron capture detection (GC/ECD) using a Hewlett-Packard 5890 Series II GC with a fused silica capillary column (DB5, 30 m, 0.25 mm, 0.25 µm; J&W Scientific, Folsom, CA). Quantitation was based on the relative response factors of individual PCB congeners and pesticides relative to the internal standard (PCB 166 by International Union of Pure and Applied Chemistry nomenclature). PCB concentrations were reported as individual congeners and as the sum of all congeners assayed (ΣPCB). The amount of each PCB congener in samples was corrected by the amount of that analyte in the procedural blank associated with the analytical batch. We did not adjust results for surrogate recoveries.

The PCB and \( p,p' \)-DDE concentrations were adjusted for total serum lipids and are expressed in units of nanograms per gram total lipids. Consistent with findings in other populations, the levels of total target PCB congeners and \( p,p' \)-DDE in the serum samples were log normally distributed. PCB congeners 118, 138, and 153 were especially of interest because they are prevalent in human serum, and the limited human data suggest that they may be associated with altered sperm motility (6).

Quality assurance and quality control. We validated the serum extraction procedure before beginning sample analysis by analyzing eight replicate samples of pooled serum fortified with target analytes at 0.02 ng/g serum. The mean ± SD percent recovery of all PCB congeners added to the serum matrix was 102 ± 13% and ranged from 73 to 125%.

Method detection limits (MDLs) were determined as three times the standard deviation obtained from the analysis of the eight serum samples fortified with target analytes, as recommended in U.S. EPA method (29).

Table 1. Subject demographics (n = 29).

<table>
<thead>
<tr>
<th>Number (%)</th>
<th>Mean ± SD</th>
<th>Minimum–maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>—</td>
<td>33 ± 4.6</td>
</tr>
<tr>
<td>Abstinence time (days)</td>
<td>—</td>
<td>3.5 ± 2.2</td>
</tr>
<tr>
<td>Current smokers</td>
<td>3 (10)</td>
<td>—</td>
</tr>
<tr>
<td>Ex-smokers</td>
<td>3 (10)</td>
<td>—</td>
</tr>
<tr>
<td>Never smokers</td>
<td>23 (80)</td>
<td>—</td>
</tr>
<tr>
<td>Current drug (cocaine) use</td>
<td>0 (0)</td>
<td>—</td>
</tr>
<tr>
<td>Ex-drug (cocaine) use</td>
<td>9 (30)</td>
<td>—</td>
</tr>
</tbody>
</table>

Table 2. Distributions of \( p,p' \)-DDE, PCB 118, PCB 138, PCB 153, and ΣPCB among the 29 study participants.a

<table>
<thead>
<tr>
<th>Mean ± SD</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>( p,p' )-DDE</td>
<td>33.4 ± 10.2</td>
<td>31.5</td>
<td>19.2</td>
</tr>
<tr>
<td>PCB 118</td>
<td>14.6 ± 8.3</td>
<td>12.5</td>
<td>6.3</td>
</tr>
<tr>
<td>PCB 138</td>
<td>26.2 ± 15.7</td>
<td>25.2</td>
<td>7.8</td>
</tr>
<tr>
<td>PCB 153</td>
<td>41.3 ± 18.8</td>
<td>39.3</td>
<td>12.5</td>
</tr>
<tr>
<td>Sum of PCB congeners</td>
<td>203 ± 78.1</td>
<td>201</td>
<td>67.7</td>
</tr>
</tbody>
</table>

a Adjusted for total serum lipids (nanogram per gram lipids).
The MDL values for all PCB congeners and HCB in serum were all < 0.05 ng/g, with most of the congeners < 0.01 ng/g. The MDL for p,p′-DDE was higher, but only because unfortified serum had high p,p′-DDE concentrations at 6.3 ng/g.

Background contamination in both batches was determined by procedural blanks. The mean ± SD for the ΣPCB was 0.28 ± 0.14 ng/g.

We evaluated analytical accuracy, precision, and extraction efficiency by analyzing two pairs (one pair in each batch) of matrix spike samples (aliquots of pooled serum spiked with 0.15 ng/g of each target analyte and two surrogate compounds, #11 and #112, added to each sample at 0.4 ng/g). The mean ± SD percent recovery for matrix spike samples was 92 ± 3.6% for ΣPCB and 86 ± 7.4% for HCB. Matrix spike percent recoveries for p,p′-DDE were inconsistent because unfortified serum had high p,p′-DDE concentrations (e.g., 6.3 ng/g) relative to the amount of p,p′-DDE added (0.15 ng/g) from the spiking solution. The mean ± SD percent recovery for two surrogate compounds, #11 and #112, were 99 ± 1.8 ±SD percent recovery for two surrogate compounds, #11 and #112, were 99 ± 1.8 ±SD percent recovery for two surrogate compounds, #11 and #112, were 99 ± 1.8 and 101 ± 4.2, respectively. Precision, expressed as mean relative percent difference (RPD) between matrix spike duplicates, was 4.3 ± 2.7.

In the blinded analysis of 10 split, unfortified samples, the within-sample (within-run) coefficient of variation (CV) for ΣPCB was 7.5%; CVs for p,p′-DDE and HCB were 4.7% and 6.2%, respectively.

### Results

Of the 32 men asked to participate, 29 (90%) agreed to participate. The mean ± SD age of the 29 participants was 33 ± 4.6 years (range, 25–45 years). Three (10%) currently smoked and 23 (80%) were never smokers (Table 1). The mean ± SD abstinence time was 3.5 ± 2.2 days (range, 0–10 days).

Of the 29 subjects, 3 (10%) had sperm concentrations < 20 million/mL, 7 (24%) had < 50% motile sperm, 9 (31%) had < 4% normal morphology, and 21 (72%) had < 14% normal morphology. A subject may be below normal in one or more semen parameters; this will not introduce bias or alter the power of the study to detect an association between exposure and outcome, although care will be needed to account for multiple comparisons. Six subjects (21%) in the pilot study were below normal in more than one semen parameter. Eighteen subjects (62%) were normal for spermatozoa concentration, motility, and morphology. These eighteen subjects were used as the comparison subjects.

#### Distribution of PCBs and p,p′-DDE. It is notable that the levels of p,p′-DDE in serum were generally higher than the levels of individual PCB congeners, which is also consistent with other studies (6). In Table 2, the distributions of p,p′-DDE, PCBs 118, 138, 153, and ΣPCB are described by their means and standard deviations, medians, and minimum and maximum values.

The mean ± SD of the ΣPCB was 203 ± 78.1 ng/g lipids; the values ranged from 67.7 to 358 ng/g lipids, approximately a 5-fold range. PCB congeners 118, 138, and 153 also showed about a 5- to 10-fold variation, and their distributions were generally more skewed than the distribution of the ΣPCB.

Associations described previously between the sum of PCB concentrations and specific PCB congeners (30,31) were confirmed in these analyses. The correlations between individual congeners, ΣPCB, and p,p′-DDE are shown in Table 3. Specifically, a strong correlation was observed between the sum of PCB concentrations and congener 153 (r = 0.92, p < 0.05) and between congeners 153 and 138 (r = 0.98, p < 0.05). The strong correlations between specific congeners and the sum of congeners (r > 0.7) make it difficult, though not impossible, to determine individual effects of a given congener.

In Table 4, the serum concentrations of p,p′-DDE, PCBs 118, 138, 153, and ΣPCB for subjects with below normal spermatozoa concentration, motility, or morphology (< 4%) are presented. Because below normal values for semen parameters may be correlated, we used subjects that were normal in all three semen parameters (sperm concentration, motility, and morphology) as the comparison group.

The data in Table 4 show that subjects with below normal concentration, motility, and morphology tend to have higher concentrations (both means and medians) of p,p′-DDE, PCBs 118, 138, 153, and ΣPCB than subjects normal in all three semen parameters. For instance, the mean ± SD values for PCB 118 for subjects with below normal sperm concentration, below normal motility, or below normal morphology, as compared to those subjects above normal in all three parameters, was 20.9 ± 11.1, 20.6 ± 4.8, 19.6 ± 4.2, and 12.8 ± 1.5 ng/g lipids, respectively. Given the small sample size in the study, we chose to present the data in tables.
and figures, thereby allowing the reader to view suggestive trends. We believe that the number of subjects in the pilot study was too small to warrant any formal statistical analysis of the data set. For all three semen quality outcomes (i.e., sperm concentration, motility, and morphology), the distribution of potential confounders (i.e., subject age and length of sexual abstinence before semen sample collection) did not differ significantly between individuals with below and above normal semen parameters. The abstinence time for the below normal spermatozoa group, below normal spermatozoa motility group, below normal morphology group, and the above normal group were 5.7 days (range, 3–10), 4.8 days (3–10), 4.3 days (2–10), and 3.2 days (0–7), respectively. The above normal group had the shortest average abstinence time, indicating that abstinence time is not a likely explanation for the higher semen parameters in this group. The age distribution for the below normal spermatozoa group, below normal spermatozoa motility group, below normal morphology group, and the above normal group were remarkably similar: 34.7 years (range, 25–45), 35.0 years (29–45), 33.4 years (25–45), and 33.1 years (27–43), respectively.

In Figures 1 and 2, individuals with below normal motility (< 50% motile) are compared to individuals that were above normal in all three semen parameters. The figures show that a) there is a higher mean concentration of PCB 118 and ΣPCB for individuals with below normal motility as compared to individuals with normal semen parameters, and b) there is a wider distribution of exposure concentrations among individuals with below normal motility as compared to normal individuals. For example, the mean serum concentration of PCB 118 among the seven men with abnormal motility was 20.6 ng/g lipid as compared to 12.8 ng/g lipid for the 18 men with above normal motility, concentration, and morphology.

**Discussion**

This study presents the results from a pilot study on the relationship between serum PCBs and p,p’-DDE and semen quality in 29 subjects. Because the number of subjects in this pilot study was too small to warrant statistical analysis of the data set, the data were presented as means and medians, comparing subjects with abnormal semen parameters to subjects with normal semen parameters. The comparison subjects were individuals with normal semen analysis results for concentration, motility, and morphology. The results show trends that are suggestive of an association between PCBs and p,p’-DDE and abnormal sperm count, motility, and morphology. These trends justified further investigation in a full-scale hypothesis-based study, currently in progress.

The results of the pilot study show that mean serum levels of PCBs were higher among individuals with below normal motility as compared to comparison subjects with normal sperm concentration, motility, and morphology. Among individuals with below normal motility, there was a wider distribution of exposure concentrations as compared to the comparison subjects. The trends noted for motility were also generally evident for morphology; individuals with below normal morphology (< 4%) had a wider distribution of PCB concentrations and higher serum PCB concentrations, on average, than comparison subjects. The data for sperm concentration (i.e., sperm counts) also showed that individuals with below normal sperm concentration had higher serum concentrations of PCBs and p,p’-DDE than comparison subjects. However, the standard errors were large because the number of subjects was small. For all three semen quality parameters, the distribution of potential confounders (i.e., subject age and length of sexual abstinence before semen sample collection) were similar between individuals with below normal parameters and comparison subjects.

We chose not to perform formal statistical analyses due to the small sample size in the pilot study. However, we believe that our data adequately summarize the results, and we did not want to place undue emphasis on p-values or confidence intervals generated in statistical tests. A full-scale study will allow us to perform formal statistical analyses and control for potential confounders.

The study subjects were recruited from MGH, which is both a community hospital and a tertiary health care facility. The community character of the hospital has further developed as a result of the recent changes in health care. The hospital owns three urban health care centers and more than 100 primary care practices, making it the largest hospital-affiliated primary care network in eastern Massachusetts. These characteristics ensure that the hospital serves a heterogeneous patient population that is representative of eastern Massachusetts. A review of the MGH Andrology Laboratory patient visits for the year before this study showed that the patient population consisted of men from a wide range of occupations (i.e., blue- and white-collar jobs) and levels of socioeconomic status. The patient area for the andrology laboratory includes neighborhoods in and around Boston. The patient population is also racially diverse. These characteristics and attributes of the MGH Andrology Laboratory population help ensure that the study’s results are generalizable.

The recruitment of men from the MGH Andrology Laboratory is unlikely to introduce selection bias because it is unlikely that participation in the study is dependent on exposure; serum PCBs and p,p’-DDE were determined only after entrance into study. We also believe it is also unlikely that the relationship between semen quality and PCBs and p,p’-DDE in this clinic population differs from the relationship in the general population.

Several researchers have hypothesized that chemicals with estrogen-like characteristics, such as PCBs and DDT, are endocrine disrupting and may adversely affect male reproduction and lead to lower sperm counts (1,2). Currently, the data in humans are limited, but several animal studies suggest that these hypotheses are biologically plausible. These chemicals, which readily penetrate the blood–testis barrier, may directly affect...
sperrmatogenesis (6,32). Effects at the mitotic or meiotic level may lead to decreased spermmatozoa production, and concentration of the chemicals in male accessory glands and seminal fluid may lead to impaired spermmatozoa motility (32). The estrogen-like characteristics of PCBs are supported by evidence showing that PCB metabolites bind to estrogen receptors (33). Jansen et al. (34) hypothesized that the adverse reproductive effects of PCBs may result from PCB congeners increasing gonadotropin-releasing hormone or affecting production and release of luteinizing hormone from the pituitary. Kelce et al. (7) showed that p,p’-DDE has antiandrogenic and estrogenic properties and may affect spermatogenesis through its antiandrogenic activity. It is currently unclear whether these mechanisms operate in humans and whether general population background levels of these compounds can alter semen quality in men.

In one of the few published human studies, Bush et al. (6) analyzed 170 semen samples for PCBs and p,p’-DDE from fertile men, men with idiopathic oligospermia, and men after vasectomy. The mean of the total PCBs (sum of all congeners) in the semen samples was 5.8 ng/g (SEM 0.8). The authors stated that these concentrations were minimal and consistent with levels seen in the general population; the semen sample PCB concentrations were of comparable concentration to residues in human blood. Bush et al. (6) found that in samples with a sperm count < 20 million cells/mL, there was a significant inverse relationship between sperm motility and the concentration of PCB congeners 153, 138, and 118 (2,4,5,2’,4’,5’-hexachlorobiphenyl and 2,4,5,3’,4’-pentachlorobiphenyl, respectively). These three congeners are major components of Aroclor 1254 and 1260. The magnitude of the effect on motility was large: It ranged from 46% to 100%, and therefore any of the maximum concentrations of the three congeners would produce complete lack of motility. Bush et al. (6) concluded that the relationship between specific PCB congeners and motility is both significant and biologically important. The findings in this study are both intriguing and disturbing because the three congeners found to be inversely predictive of sperm motility are ubiquitous in the human population (35). Further evidence of a possible relationship between PCBs and semen quality is presented in a study in rats which suggests that PCB exposure affects the ability of sperm to fertilize eggs (36).

The current and ongoing research on temporal trends in sperm concentration emphasize the need for a better understanding of the relationship between environmental exposures and semen quantity and quality. Hypothesized environmental exposures of interest include PCBs and pesticides. Our full-scale study is currently in progress and is designed to investigate the relationship between environmental exposures and tests function. The study includes assessments of semen quantity and quality, as well as measurements of reproductive hormones.

REFERENCES AND NOTES