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The Relationship between Human Semen Parameters and Environmental Exposure to Polychlorinated Biphenyls and *p,p′*-DDE

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Scientific and public concern exists about potential reproductive health effects of persistent chlorinated organic chemicals, such as polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), and dichlorodiphenyldichloroethylene (DDE, the most stable daughter compound of DDT). To explore the hypothesis that environmental exposures to PCBs and DDE are associated with altered semen parameters, we conducted a cross-sectional study of 212 male partners of subfertile couples who presented to the Massachusetts General Hospital Andrology Laboratory. Semen parameters were analyzed as both a continuous measure and dichotomized based on World Health Organization reference values for sperm concentration (< 20 million/mL), motility (< 50% motile), and Kruger strict criteria for morphology (< 4% normal). The comparison group for the dichotomized analysis was men with all three semen parameters above the reference values. In serum, 57 PCB congeners and *p,p′*-DDE were measured by congener-specific analysis using gas chromatography with electron capture detection. There were dose–response relationships among PCB-138 and sperm motility (odds ratio per tertile, adjusted for age, abstinence, and smoking, and *p*-value for trend were, respectively, 1.00, 1.68, 2.35, and *p*-value = 0.03) and morphology (1.00, 1.36, 2.53, *p*-value = 0.04). There was limited evidence of an inverse relationship between sum of PCBs, as well as those PCBs classified as cytochrome P450 enzyme inducers, with sperm motility and sperm morphology, as well as limited evidence of an inverse association between *p,p′*-DDE and sperm motility. The lack of a consistent relationship among semen parameters and other individual PCB congeners and groupings of congeners may indicate a difference in spermatotoxicity between congeners. *Key words:* DDT, environmental health, polychlorinated biphenyls, reproductive health, semen, sperm. *Environ Health Perspect* 111:1505–1511 (2003). doi:10.1289/ehp.6175 available via *http://dx.doi.org/* [Online 19 May 2003]

Currently scientific and public concern exists about persistent organic chemicals, such as polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), and dichlorodiphenyldichloroethylene (DDE, the most stable daughter compound of DDT). Several researchers have hypothesized that these compounds may be associated with the suggested, although not confirmed, downward trend in semen parameters (Irvine et al. 1996; Sharpe and Skakkebaek 1993). The concern stems from studies showing that PCBs and *p,p′*-DDE are found in a large proportion of the general population (CDC 2003; Longnecker et al. 1997; Murphy and Harvey 1985; Stehr-Green 1989) and from animal and limited human studies suggesting possible associations of exposure to PCBs and *p,p′*-DDE with semen abnormalities (Bush et al. 1986; Dallinga et al. 2002; Richthoff et al. 2003). Serum levels of PCBs and *p,p′*-DDE are an integrated measure of internal dose, reflecting exposure from all sources over the previous years; depending on congener type, the half-lives of PCBs in the blood range from 1 to 10 or more years, whereas *p,p′*-DDE has a half-life of 10 or more years (Brown 1994; Phillips et al. 1989b).

PCBs and *p,p′*-DDE are persistent lipophilic chemicals. DDT was widely used as an insecticide, whereas PCBs were used in cutting oils and lubricants and as electrical insulators. Although their use and manufacture were banned nearly 30 years ago, they are ubiquitous and persist in the environment. They are distributed worldwide as environmental pollutants and have been measured in air, water, aquatic and marine sediments, fish, and wildlife (De Voogt and Brinkman 1989). 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 foods (fish, meat, eggs, and dairy products) and water, as well as through dermal contact (soil and house dust) and inhalation (indoor air in buildings that have various sources, as well as outdoor air).

Studies suggest that there is a temporal downward trend in human semen quality (Auger et al. 1995; Carlsen et al. 1992; Giwercman et al. 1993; Irvine et al. 1996; Swan et al. 1997, 2000). However, other studies suggest that semen quality has not declined or may have even increased marginally (Bujan et al. 1996; Fisch et al. 1996; Paulsen et al. 1996; Sherins 1995). Nevertheless, most of these studies suggest that semen quality varies by geographic location (Fisch and Goluboff 1996). It has been hypothesized that the geographic variation in semen quality may be caused by environmental exposures, lifestyle factors, or some unknown cause(s) (Fisch and Goluboff 1996). However, the temporal trend studies lacked information at the individual level on lifestyle factors, such as cigarette smoking, as well as information on exposure to potential environmental contaminants, such as PCBs and DDT (Sun et al. 1996; Vine et al. 1994).

To determine whether environmental levels of PCBs and *p,p′*-DDE are associated with altered semen parameters in adult men, we selected a study population without specific exposure to these compounds. Detecting even an association of small magnitude may have large public health significance because of the widespread distribution of PCBs and *p,p′*-DDE in the general population.

Materials and Methods

Subjects. The study was approved by the Harvard School of Public Health and Massachusetts General Hospital (MGH) Human Subjects Committees, and all subjects signed an informed consent. Subjects were male partners of subfertile couples who presented to the Vincent Burnham Andrology Laboratory at MGH between January 2000 and October 2001 for semen analysis. Individual men may or may not have been infertile. Sixty-six percent of eligible men between 20 and 54 years old agreed to participate. Men presenting for postvasectomy semen analysis and men receiving treatment for infertility, such as hormonal treatments, were excluded. Height and weight were measured, and a questionnaire was used to collect information on medical history and lifestyle factors.

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We thank I. Schiff, T. Toth, K. Isaacson, J. Shifren, and L. Godfrey-Bailey; R. Stolyar and S. Forsberg for the chemical analyses; and A. Trisini for assistance with manuscript preparation.

This study was supported by grants ES09718 and ES00002 from the National Institute of Environmental Health Sciences.

The authors declare they have no conflict of interest.

Received 24 December 2002; accepted 19 May 2003.

Semen analysis. Each man produced a single semen sample by masturbation into a sterile plastic specimen cup. The sample was liquefied at 37°C for 20 min before analysis. Subjects were instructed to abstain from ejaculation for 48 hr before producing the semen sample and to complete a questionnaire on the length of the sexual abstinence period.

Semen analyses were performed without knowledge of subjects' PCB and *p,p′*-DDE levels. All semen samples were analyzed for sperm concentration and motion parameters by computer-aided sperm analysis (CASA; version 10HTM-IVOS, Hamilton-Thorn, Beverly, MA). Setting parameters and the definition of measured sperm motion parameters for CASA were established by Hamilton-Thorn (frames acquired, 30; frame rate, 60 Hz; straightness threshold, 80.0%; medium average path velocity cutoff, 25.0 μm/sec; duration of the tracking time, 0.38 sec). To measure both sperm concentration and motility, we placed aliquots of semen samples (5 μL) into a prewarmed (37°C) Makler counting chamber (Sefi Medical Instruments, Haifa, Israel). A minimum of 200 sperm from at least four different fields were analyzed from each specimen. Percent motile sperm was defined as World Health Organization (WHO) "A" grade sperm (rapidly progressive with a velocity ≥ 25 μm/sec at 37°C) plus "B" grade sperm (slow/sluggish progressive with a velocity < 5 μm/sec but < 25 μm/sec).

Using the "feathering" method from the WHO (1999), we made at least two slides for each fresh semen sample. The resulting thin smear was allowed to air dry for 1 hr before staining with a Diff-Quik staining kit (Dade Behring AG, Düdingen, Switzerland). Morphologic assessment was performed with a Nikon microscope using an oil immersion 100× objective (Nikon Company, Tokyo, Japan). Spermatozoa were assessed and scored as normal or abnormal using the strict criteria of Kruger et al. (1998). A minimum of 200 spermatozoa were counted from two slides for each specimen. Results were expressed as the percentage of normal spermatozoa.

Serum PCB and* p,p′*-DDE measurements. Blood samples were collected on the same day as the semen sample and analyzed by the Organic Chemistry Analytical Laboratory at the Harvard School of Public Health. Target analytes included 57 individual PCB congeners and *p,p′*-DDE. Details of the sampling, analytical, and quality control (QC) procedures are described elsewhere (Korrick et al. 2000). Briefly, the blood samples were collected in red-top Vacutainer tubes, and the serum fraction was separated by centrifugation. Serum samples were stored in solvent rinsed glass vials with Teflon-lined caps at –80°C until analysis.

For extraction, we used procedures developed by the Centers for Disease Control (Needham 1981) 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.

Because PCBs and *p,p′*-DDE partition according to the lipid content of tissues, and serum lipid levels vary between fasting and nonfasting states, a correction for serum lipids is needed for the valid interpretation of serum levels (Phillips et al. 1989a). Therefore, percent lipid for each serum sample was measured gravimetrically, by weighing an aliquot of sample extract evaporated to dryness. The mean (SD) for the 212 samples was 0.51% (0.20) lipid.

The serum extracts were analyzed by GC with electron capture detection (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). Confirmatory analysis was done using a Hewlett-Packard 6980 GC with a Micro-ECD (GC/μECD) and capillary column of different polarity. Quantitation was based on the response factors of individual PCB congeners and *p,p′*-DDE relative to the internal standard (PCB-166 by International Union for Pure and Applied Chemistry nomenclature; Ballschmiter et al. 1992). 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. Results were not adjusted for surrogate recoveries.

The PCB and *p,p′*-DDE concentrations were adjusted for total serum lipids and are expressed in units of ng/g total lipids. PCB congeners 118, 138, and 153, as well as *p,p′*-DDE, were especially of interest because they are prevalent in human serum and because the limited human data suggest that they may be associated with altered sperm motility (Ayotte et al. 2001; Bush et al. 1986; Dallinga et al. 2002).

Quality assurance and QC. The Organic Chemistry Analytical Laboratory follows strict QC and quality assurance (QA) procedures. The laboratory successfully participates in various intercalibration exercises, including an international intercomparison program organized by the Institute for Quality Management and Medicine, at the University of Erlangen-Nuremberg, Germany (annually), and the international Ring tests sponsored by the Arctic Monitoring and Assessment Program and organized by Quebec National Institute of Public Health, Canada (three times per year).

Method detection limit (MDL) values were determined as three times the SD obtained from the analysis of the eight aliquots of pooled serum fortified with target analytes at 0.02 ng/g serum, as recommended by U.S. Environmental Protection Agency methods (U.S. EPA 1984). The MDL values for all PCB congeners were < 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 13 analytical batches was determined by procedural blanks. The mean (SD) for ΣPCB was 0.18 (0.02) ng/g. Analytical accuracy, precision, and extraction efficiency were evaluated by the analyses of two pairs (one pair in each batch) of matrix spike samples (aliquots of pooled bovine serum spiked at 0.24 ng/g of each PCB congener, 0.13 ng/g of *p,p′*-DDE) and two surrogate compounds, PCB-30 and PCB-112, added to each sample at 0.67 ng/g. The mean (SD) percent recovery for matrix spike samples was 95% (6.6) for ΣPCB and 99% (10) for DDE. The mean (SD) percent recovery for two surrogate compounds, PCB-30 and PCB-112, was 96% (6.8) and 98% (4.1), respectively. Precision, expressed as mean relative percent difference between ΣPCBs in matrix spike duplicates, was 5.5% (4.8).

Statistical analysis. Statistical Analysis Software (SAS), version 8.2 (SAS Institute Inc., Cary, NC), was used for data analysis. On the basis of earlier studies (Bush et al. 1986; Dallinga et al. 2002; Richthoff et al. 2003), we explored the relationship between semen parameters and three individual PCB congeners (PCBs 118, 138, and 153), as well as ΣPCBs. Analyses using the other individual PCB congeners were not conducted. In addition, an analysis of the relationship between semen parameters and groupings of PCBs, based on structural and biological activity as proposed by Wolff et al. (1997), was conducted. PCBs were grouped as follows: group 1, potentially estrogenic and weak phenobarbitol inducers (congeners 44, 49, 52, 101, 187, 174, 177, 157/201); group 2, potentially antiestrogenic and dioxin-like (congeners 95/66, 74, 77/110, 105/141, 118, 156, 167, 128, 138, 170); and group 3, phenobarbital, CYP1A, and CYP2B inducers (congeners 99, 153, 180, 196/203, 183). Relationships between semen parameters and *p,p′*-DDE were also explored.

Because the distributions of the PCBs and *p,p′*-DDE were not normally distributed, nonparametric methods (Spearman correlation coefficients or Wilcoxon signed rank tests) were used to explore their relationships with age, body mass index (BMI), smoking status, and race. Chi-square tests were used to explore the relationships between categorical semen parameters and categorical covariates, such as abstinence time, race, and smoking status.

In preliminary analyses, we used scatterplots and multiple linear regression to explore the relationships among semen parameters and PCBs and *p,p′*-DDE. Because sperm

concentration was highly skewed and the residuals were not normally distributed, they were log-transformed after a small positive number was added (Berman et al. 1996). For our primary analyses, we conducted multivariate logistic regression analyses in which semen parameters were dichotomized based on WHO (1999) reference values for sperm concentration (< 20 million/mL) and motility (< 50% motile sperm) and Kruger strict criteria for morphology (< 4% normal sperm). The comparison group was defined as men with all three semen parameters at or above the reference value. In these analyses, we divided PCBs and *p,p′*-DDE into tertiles, which were used as dummy variables to allow for the exploration of nonlinear relationships. A separate multivariate logistic regression model was used for each semen parameter. Individuals whose values were above the WHO reference value on the semen parameter of interest, but below the WHO reference value on one or both of the other two semen parameters, were excluded from the analysis on the semen parameter of interest. For instance, in the logistic regression analysis on the relationship between sperm concentration and organochlorines, men with sperm concentrations below reference were considered "cases," whereas men with all three semen parameters above the reference value were considered "comparison" subjects. Men with sperm concentration above the reference value, but with motility and/or morphology below the reference value, were excluded from this specific analysis.

Covariates considered for inclusion in the multivariate regression and logistic models included smoking status, race, age, BMI, and abstinence time. Their final inclusion in the multivariate models was based on statistical and biologic considerations (Hosmer and Lemeshow 1989). Age was modeled as a continuous independent variable. Abstinence time was modeled as an ordinal five-category variable (≤ 2, 3, 4, 5, and ≥ 6 days). Smoking status was included as a dummy variable (current and former vs. never).

Results

Of the 212 men (66% participation) in the study population, 40 (19%) had a sperm concentration < 20 million/mL, 97 men (46%) had < 50% motile sperm, and 58 men (27%) had < 4% normally shaped sperm. Ninety-eight (46%) men had values above reference on all three semen parameters. The semen parameter categories were not mutually exclusive: A man could contribute data to any one, two, or all three of the below-reference-value groups. Overall, the subjects were primarily Caucasian (79%), with 6% African American and 8% Hispanic. The mean (SD) age was 36.0 (5.4) years. Seventy-three percent had never smoked.

The demographic distribution, by semen parameter, is summarized in Table 1. Advanced age and Hispanic ethnic origin were predictors of one or more below-reference semen parameters, whereas current cigarette smoking was not strongly associated with below-reference semen parameters. However, only eight men in the comparison group were current smokers, which limited the ability to investigate the relationship between smoking and semen quality. As expected, men who had a previous examination for infertility were more likely to be below the reference value on all three semen parameters.

We measured 57 PCB congeners and *p,p′*-DDE. There was a wide distribution of both the PCB congeners and *p,p′*-DDE concentrations (Table 2). The median *p,p′*-DDE concentration was 222 ng/g lipids, with a range from 64.2 to 8,912 ng/g lipid. The median of ΣPCBs was 216 ng/g lipid and ranged from 56.0 to 1,733 ng/g lipid. The levels of *p,p′*-DDE in serum were higher than the levels of individual PCB congeners, which is also consistent with other studies (Bush et al. 1986).

Associations described previously among ΣPCBs and specific PCB congeners were confirmed in these analyses (De Voto et al 1997; Koopman-Esseboom et al. 1994). Strong correlations were observed between ΣPCBs with PCB congeners 153 and 138, and with the three groupings of PCBs ($r > 0.9$, p-values < 0.0001). There was a moderate correlation between ΣPCBs and PCB-118 ($r = 0.6$, p-value < 0.0001). The three groupings of PCB congeners were strongly correlated with each other ($r > 0.8$, p-values < 0.0001). Among the individual congeners, PCB-153 and PCB-138 were strongly correlated ($r = 0.9$, p-value < 0.0001), whereas PCB-118 was moderately correlated with PCB-153 and PCB-138 ($r = 0.6$ and 0.7,

Table 1. Demographic and medical history by semen parameters ($n = 212$).

	Comparison subjects[a] ($n = 98$)	Sperm concentration (< 20 million/mL) ($n = 40$)[b]	Sperm motility (< 50% motile) ($n = 97$)[b]	Sperm morphology (< 4% normal) ($n = 58$)[b]
Age (mean ± SD)	35.3 ± 5.1	36.5 ± 6.0	36.9 ± 5.7*	36.3 ± 5.3
Abstinence time[c] [*n* (%)]				
≤ 2 days	25 (25.8)	10 (25.0)	25 (25.8)	10 (17.2)
3 days	33 (34.0)	9 (22.5)	30 (30.9)	18 (31.0)
4 days	14 (14.4)	9 (22.5)	17 (17.5)	11 (19.0)
5 days	11 (11.3)	4 (10.0)	7 (7.2)	4 (6.9)
≥ 6 days	14 (14.4)	8 (20.0)	18 (18.6)	15 (25.9)
Race[c] [*n* (%)]				
White	82 (83.7)	29 (74.4)	68 (71.6)*	44 (77.2)
African American	5 (5.1)	3 (7.7)	8 (8.4)	4 (7.0)
Hispanic	4 (4.1)	3 (7.7)	12 (12.6)*	5 (8.8)
Other	7 (7.1)	4 (10.3)	7 (7.4)	4 (7.0)
Smoking status[c] [*n* (%)]				
Never smoker	73 (74.5)	26 (66.7)	67 (69.8)	39 (68.4)
Ever smoker	25 (25.5)	13 (33.3)	29 (30.2)	18 (31.6)
Current smoker	8 (8.2)	5 (12.8)	10 (10.4)	5 (8.8)
Ex-smoker	17 (17.4)	8 (20.5)	19 (19.8)	13 (22.8)
Previous exam for infertility [*n* (%)]	19 (19.4)	19 (48.7)*	41 (42.7)*	24 (42.4)*

Wilcoxon signed rank tests were used for age comparisons across semen parameter groups. Chi-square tests were used for comparisons of categorical variables across semen parameter groups.
[a]Subjects with sperm concentration ≥ 20 million/mL, motility ≥ 50% motile, and morphology ≥ 4% normal. [b]A subject may contribute data to more than one category. [c]Race information missing on two men, smoking missing on one, and abstinence data missing on one. *p-Value < 0.05 compared with comparison group.

Table 2. Distribution of serum levels of *p,p′*-DDE, PCB-118, PCB-138, PCB-153, and ΣPCBs ($n = 212$).

		Percentile						
	Minimum	5th	25th	50th	75th	95th	Maximum	Geometric mean
p,p′-DDE	64.2	93.6	157.0	222.2	374.7	1648.2	8911.8	275.3
PCB-118	3.1	5.3	7.8	12.0	18.7	35.7	61.5	12.6
PCB-138	7.3	14.1	22.3	31.2	47.4	102.3	295.4	33.6
PCB-153	9.3	19.5	29.0	41.8	61.1	128.0	361.3	43.8
ΣPCB	56.0	116.0	152.7	215.9	306.6	568.1	1732.6	226.2
ΣEstrogenic PCBs (group 1)	3.9	7.4	10.9	15.7	22.1	43.2	204.8	16.4
ΣDioxin-like PCBs (group 2)	19.6	38.1	55.0	74.0	116.1	227.7	518.5	81.8
ΣEnzyme-inducing PCBs (group 3)	19.7	40.4	61.1	88.8	132.6	267.7	829.6	92.9

All serum levels are adjusted for lipids and expressed as ng/g lipids.

respectively, p-values < 0.0001). There were weak to moderate correlations between *p,p′*-DDE with PCB congeners 153, 138, and 118 and ΣPCBs ($0.3 < r < 0.4$, p-values < 0.0001).

There were weak to moderate correlations between age and ΣPCBs, groupings of PCBs, and PCB-138 and PCB-153 ($0.3 < r < 0.4$, p-values < 0.0001), and between *p,p′*-DDE and age ($r = 0.24$, p-value = 0.0004). There were weak and nonsignificant relationships between BMI and the PCBs, and a weak inverse significant relationship between BMI and *p,p′*-DDE ($r = -0.1$, p-value = 0.05). Except for PCB-118, there were nonsignificant relationships between smoking status and PCBs. For current smokers, the median concentration of PCB-118 was 7.8 ng/g lipid, compared with 13.1 ng/g lipid and 12.0 ng/g lipid for ex-smokers and never smokers, respectively. Hispanics had lower PCB concentrations, but higher *p,p′*-DDE concentrations, than did Caucasians. For Hispanics, the median concentration of ΣPCBs and *p,p′*-DDE was 143 ng/g lipid and 666 ng/g lipid, respectively, compared with 220 ng/g lipid and 204 ng/g lipid, respectively, for Caucasians. African Americans also had significantly higher *p,p′*-DDE, 370 ng/g lipid, compared with Caucasians, but PCB concentrations in African Americans did not differ with those measured in Hispanics and Caucasians.

In the multivariate analyses, age, smoking status, and abstinence time were included in each model because they are considered predictors of semen quality (Blackwell and Zaneveld 1992; Kidd et al. 2001; Vine et al. 1994). BMI was not included in the models because it was weakly associated with PCBs and *p,p′*-DDE and was not a known predictor of semen parameters. Crude and adjusted odds ratios (ORs) for below-reference-value semen parameters by tertiles of PCBs and *p,p′*-DDE are presented in Tables 3–5. In the adjusted analyses, there were significant dose–response relationships (OR per tertile adjusted for age, abstinence, and smoking) between PCB-138 and below-reference-value sperm motility (1.00, 1.68, 2.35, respectively; p-value for trend = 0.03) and below-reference-value sperm morphology (1.00, 1.36, 2.53, p-value for trend = 0.04). There was a nonsignificant dose–response relationship for PCB-138 and below-reference-value sperm concentration (1.00, 1.72, 1.62, p-value for trend = 0.3). The crude ORs for PCB-138 were larger than the adjusted ORs. Furthermore, the crude ORs for the relationship between sperm motility and PCB-153 and ΣPCBs (respectively: 1.00, 1.10, 1.86, p-value for trend = 0.08; 1.00 1.77, 1.88, p-value for trend = 0.08) showed a stronger dose–response trend than did the adjusted dose–response relationships. Although not statistically significant, *p,p′*-DDE showed a weak dose–response trend with below-reference-value sperm motility (1.00, 1.14, 1.51, p-value for trend = 0.3).

Crude and adjusted ORs for below-reference-value semen parameters by tertiles of groupings of PCBs are presented in Tables 6–8. There were dose–response relationships (OR per tertile adjusted for age, abstinence, and smoking) between PCB congeners in group 3 (cytochrome P450 enzyme inducers) and below-reference-value sperm motility (1.00, 1.56, 1.80, p-value for trend = 0.1) and below-reference-value sperm morphology (1.00, 1.33, 1.93, p-value for trend = 0.1) (Tables 7 and 8, respectively). The crude analysis showed a stronger and statistically significant dose–response trend for the relationship between group-3 PCBs and below-reference-value sperm motility and morphology (respectively: 1.00, 1.71, 2.12, p-value for trend = 0.04; 1.00, 1.59, 2.23, p-value for trend = 0.05). For the other two PCB groupings (group 1, potentially estrogenic; group 2, potentially antiestrogenic and dioxin-like), the ORs for the third tertile were increased for below-reference-value sperm motility and morphology, but there was limited evidence of a trend.

Table 3. Crude and adjusted ORs (95% CI) for below reference value sperm concentration (SC)[a] by tertiles of *p,p′*-DDE and PCBs.

	p,p′-DDE and PCB tertiles			*p*-Value
	Tertile 1	Tertile 2	Tertile 3	for trend
p,p′-DDE				
SC < 20 million/mL (*n*)	15	11	14	
Crude OR (95% CI)	1.00	0.73 (0.30–1.83)	1.06 (0.44–2.55)	0.9
Adjusted OR (95% CI)[b]	1.00	0.64 (0.24–1.70)	0.96 (0.37–2.51)	0.9
PCB-118				
SC < 20 million/mL (*n*)	13	16	11	
Crude OR (95% CI)	1.00	1.09 (0.45–2.62)	0.82 (0.32–2.10)	0.7
Adjusted OR (95% CI)[b]	1.00	1.01 (0.39–2.66)	0.78 (0.28–2.13)	0.6
PCB-138				
SC < 20 million/mL (*n*)	11	15	14	
Crude OR (95% CI)	1.00	1.71 (0.69–4.22)	1.96 (0.77–4.97)	0.1
Adjusted OR (95% CI)[b]	1.00	1.72 (0.68–4.38)	1.62 (0.59–4.46)	0.3
PCB-153				
SC < 20 million/mL (*n*)	14	10	16	
Crude OR (95% CI)	1.00	0.74 (0.29–1.87)	1.52 (0.64–3.65)	0.4
Adjusted OR (95% CI)[b]	1.00	0.58 (0.21–1.60)	1.24 (0.47–3.24)	0.7
ΣPCB congeners				
SC < 20 million/mL (*n*)	14	13	13	
Crude OR (95% CI)	1.00	1.18 (0.48–2.88)	1.18 (0.48–2.88)	0.7
Adjusted OR (95% CI)[b]	1.00	1.00 (0.39–2.60)	0.89 (0.33–2.38)	0.8

Tertile cut points (ng/g lipid): *p,p′*-DDE: 64.2–184.3, 184.9–296.6, 302.5–8911.8; PCB-118: 3.1–9.1, 9.2–16.3, 16.5–61.5; PCB-138: 7.3–24.9, 25.0–38.5, 39.3–295.4; PCB-153: 9.3–32.3, 32.4–52.9, 53.5–361.3; ΣPCBs: 56.0–175.1, 177–260.8, 265.1–1732.6.
[a]Below reference value for sperm concentration (SC) was defined as < 20 million sperm/mL. [b]Adjusted for age (continuous), abstinence time (five categories: ≤ 2, 3, 4, 5, and ≥ 6 days) and smoking (current, former, and never).

Table 4. Crude and adjusted ORs (95% CI) for below reference value sperm motility (SM)[a] by tertiles of *p,p′*-DDE and PCBs.

	p,p′-DDE and PCB tertiles			*p*-Value
	Tertile 1	Tertile 2	Tertile 3	for trend
p,p′-DDE				
SM < 50% motile (*n*)	27	31	39	
Crude OR (95% CI)	1.00	1.15 (0.57–2.32)	1.64 (0.82–3.28)	0.2
Adjusted OR (95% CI)[b]	1.00	1.14 (0.55–2.35)	1.51 (0.73–3.13)	0.3
PCB-118				
SM < 50% motile (*n*)	33	30	34	
Crude OR (95% CI)	1.00	0.81 (0.40–1.61)	1.00 (0.50–1.99)	1.00
Adjusted OR (95% CI)[b]	1.00	0.75 (0.36–1.55)	0.94 (0.46–1.94)	0.9
PCB-138				
SM < 50% motile (*n*)	24	33	40	
Crude OR (95% CI)	1.00	1.72 (0.85–3.47)	2.56 (1.26–5.20)	0.009
Adjusted OR (95% CI)[b]	1.00	1.68 (0.82–3.42)	2.35 (1.11–4.99)	0.03
PCB-153				
SM < 50% motile (*n*)	28	30	39	
Crude OR (95% CI)	1.00	1.10 (0.55–2.21)	1.86 (0.93–3.73)	0.08
Adjusted OR (95% CI)[b]	1.00	0.93 (0.45–1.94)	1.60 (0.75–3.41)	0.2
ΣPCB congeners				
SM < 50% motile (*n*)	25	35	37	
Crude OR (95% CI)	1.00	1.77 (0.88–3.58)	1.88 (0.93–3.77)	0.08
Adjusted OR (95% CI)[b]	1.00	1.54 (0.74–3.22)	1.60 (0.76–3.39)	0.2

Tertile cut points (ng/g lipid): *p,p′*-DDE: 64.2–184.3, 184.9–296.6, 302.5–8911.8; PCB-118: 3.1–9.1, 9.2–16.3, 16.5–61.5; PCB-138: 7.3–24.9, 25.0–38.5, 39.3–295.4; PCB-153: 9.3–32.3, 32.4–52.9, 53.5–361.3; ΣPCBs: 56.0–175.1, 177–260.8, 265.1–1732.6.
[a]Below reference value for sperm motility (SM) was defined as < 50% motile sperm. [b]Adjusted for age (continuous), abstinence time (five categories: ≤ 2, 3, 4, 5, and ≥ 6 days) and smoking (current, former, and never).

The interpretation of the results from the multivariate regression analyses, in which semen parameters and PCBs and *p,p′*-DDE were used as continuous measures, was generally similar to that for the logistic regression analyses. After adjustment for age, abstinence time, and smoking status, there was a statistically significant inverse relationship between log-transformed sperm concentration and PCB-138 [β = –24.8 million sperm per mL/interquartile range (IQR); 95% confidence interval (95% CI), –24.6 to –25.0; $p < 0.008$), and an inverse, although not statistically significant, relationship between PCB-138 and motility (β = –1.8% motility/IQR; 95% CI, –4.4 to 0.87; $p = 0.2$) and morphology (β = –0.28% morphology/IQR; 95% CI, –0.78 to 0.23; $p = 0.3$). In comparison, in the logistic regression analyses, PCB-138 was statistically significantly inversely associated with sperm motility and morphology but nonsignificantly inversely associated with sperm concentration. Although statistical significance does differ, the dose–response relationships from the logistic and regression analyses consistently show inverse relationships between PCB-138 and semen parameters. The relationships observed between semen parameters and *p,p′*-DDE and individual PCB congeners, ΣPCBs, and groupings of PCBs were consistent with the interpretation of the results of the logistic regression analyses.

We conducted sensitivity analyses after excluding nine men with azoospermia to prevent undue statistical influence from extreme sperm counts (i.e., zero) and because the mechanism responsible for azoospermia may be related to an obstructive mechanism or Y-chromosome deletions. In the reanalysis, the results remained essentially unchanged (data not shown).

Table 5. Crude and adjusted ORs (95% CI) for below reference value sperm morphology (SMPH)[a] by tertiles of *p,p′*-DDE and PCBs.

	p,p′-DDE and PCB tertiles			*p*-Value
	Tertile 1	Tertile 2	Tertile 3	for trend
p,p′-DDE				
SMPH < 4% normal (*n*)	17	24	17	
Crude OR (95% CI)	1.00	1.41 (0.65–3.09)	1.13 (0.49–2.61)	0.8
Adjusted OR (95% CI)[b]	1.00	1.28 (0.56–2.93)	1.14 (0.47–2.80)	0.8
PCB-118				
SMPH < 4% normal (*n*)	17	20	21	
Crude OR (95% CI)	1.00	1.04 (0.47–2.34)	1.20 (0.53–2.69)	0.7
Adjusted OR (95% CI)[b]	1.00	0.94 (0.40–2.25)	1.10 (0.47–2.61)	0.8
PCB-138				
SMPH < 4% normal (*n*)	15	17	26	
Crude OR (95% CI)	1.00	1.42 (0.61–3.27)	2.67 (1.19–5.96)	0.02
Adjusted OR (95% CI)[b]	1.00	1.36 (0.57–3.22)	2.53 (1.06–6.03)	0.04
PCB-153				
SMPH < 4% normal (*n*)	18	15	25	
Crude OR (95% CI)	1.00	0.86 (0.37–1.96)	1.85 (0.85–4.06)	0.1
Adjusted OR (95% CI)[b]	1.00	0.65 (0.26–1.59)	1.64 (0.69–3.86)	0.2
ΣPCB congeners				
SMPH < 4% normal (*n*)	17	17	24	
Crude OR (95% CI)	1.00	1.27 (0.56–2.89)	1.79 (0.82–3.92)	0.1
Adjusted OR (95% CI)[b]	1.00	1.02 (0.42–2.45)	1.56 (0.67–3.63)	0.3

Tertile cut points (ng/g lipid): *p,p′*-DDE: 64.2–184.3, 184.9–296.6, 302.5–8911.8; PCB-118: 3.1–9.1, 9.2–16.3, 16.5–61.5; PCB-138: 7.3–24.9, 25.0–38.5, 39.3–295.4; PCB-153: 9.3–32.3, 32.4–52.9, 53.5–361.3; ΣPCBs: 56.0–175.1, 177–260.8, 265.1–1732.6.
[a]Below reference value for morphology (SMPH) was defined as < 4% normally shaped sperm. [b]Adjusted for age (continuous), abstinence time (five categories: ≤ 2, 3, 4, 5, and ≥ 6 days) and smoking (current, former, and never).

Table 6. Crude and adjusted ORs (95% CI) for below reference value sperm concentration (SC)[a] by tertiles of grouped PCBs.

	Tertiles of PCB congener groupings			*p*-Value
	Tertile 1	Tertile 2	Tertile 3	for trend
ΣEstrogenic PCBs (group 1)				
SC < 20 million/mL (*n*)	12	15	13	
Crude OR (95% CI)	1.00	1.45 (0.59–3.56)	1.26 (0.50–3.16)	0.6
Adjusted OR (95% CI)[b]	1.00	1.47 (0.57–3.76)	1.01 (0.37–2.76)	0.9
ΣDioxin-like PCBs (group 2)				
SC < 20 million/mL (*n*)	12	14	14	
Crude OR (95% CI)	1.00	1.17 (0.47–2.88)	1.46 (0.58–3.65)	0.4
Adjusted OR (95% CI)[b]	1.00	1.05 (0.41–2.71)	1.22 (0.46–3.25)	0.7
ΣEnzyme-inducing PCBs (group 3)				
SC < 20 million/mL (*n*)	13	12	15	
Crude OR (95% CI)	1.00	1.16 (0.47–2.90)	1.61 (0.66–3.90)	0.3
Adjusted OR (95% CI)[b]	1.00	1.06 (0.41–2.76)	1.23 (0.46–3.32)	0.7

Tertile cut points (ng/g lipid): ΣEstrogenic PCBs: 3.9–12.5, 12.6–19.4, 19.5–204.8; ΣDioxin-like PCBs: 19.6–61.0, 61.4–94.1, 94.9–518.5; ΣEnzyme-inducing PCBs: 19.7–68.6, 69.7–111.1, 114.5–829.6.
[a]Below reference value for sperm concentration (SC) was defined as < 20 million sperm/mL. [b]Adjusted for age (continuous), abstinence time (five categories: ≤ 2, 3, 4, 5, and ≥ 6 days) and smoking (current, former, and never).

Discussion

In the present study, we found dose–response relationships between PCB-138 and decreased sperm concentration, sperm motility, and poor sperm morphology measured using the strict criteria. There were inverse, although nonsignificant, relationships of ΣPCBs and group 3 PCBs (enzyme inducers) with sperm motility and sperm morphology. Group 3 did not include PCB-138, which was in group 2. There was weak evidence of an association between *p,p′*-DDE and sperm motility. Overall, these results suggest that individual congeners and groupings of congeners based on structure–activity may represent a more appropriate approach to the analysis between PCBs and health end points. As widely discussed in the literature, individual congeners have different toxicities and biologic activities (Ahlborg et al. 1994; Hansen 1999; Safe 1990, 1993). Depending on the number and pattern of the chlorine substitutions, the biologic activity of individual PCB congeners will vary. Therefore, in addition to conducting analyses using individual PCB congeners and ΣPCBs, we used congener groupings based on both structure and potential biologic activity based on groupings proposed by Wolff et al. (1997). Although these groupings represent an improvement in exposure classification compared with earlier studies that only used ΣPCBs, they still have several limitations. For instance, within groups, congeners are summed using concentration, but no weighting factor is applied to account for differential activities. In addition, these groupings are based on PCB activity in animal and *in vitro* systems, and not in humans. Finally, the groupings are not based specifically on potential testicular toxicity in animals or humans, but rather on general potential biologic activity. These limitations will contribute to exposure misclassification of the groupings of PCBs. Although the direction of the misclassification bias is unknown, it is generally expected to bias toward the null hypothesis.

Some animal data suggest that PCBs and *p,p′*-DDE may be hormonally active and therefore adversely affect semen parameters. These compounds, which readily penetrate the blood–testis barrier, may directly affect spermatogenesis (Bush et al. 1986; Tuohimaa and Wichmann 1985). Effects at the mitotic or meiotic level may lead to decreased sperm production, whereas the targeting of postmeiotic processes and epididymal sperm maturation may lead to impaired sperm motility. The estrogen-like characteristics of specific PCBs are supported by evidence showing that PCB metabolites bind to estrogen receptors (Korach et al. 1988). Jansen et al. (1993) 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. (1995) showed that *p,p′*-DDE has antiandrogenic and estrogenic properties and may affect spermatogenesis through its antiandrogen activity.

In one of the several published human studies, Bush et al. (1986) analyzed 170 semen samples for PCBs and *p,p′*-DDE from fertile men, men with idiopathic oligospermia, and men postvasectomy. The mean (SE) of ΣPCBs in the semen samples was 5.8 ng/g (0.8). The authors stated that these concentrations were consistent with levels seen in the general population; the semen sample PCB concentrations were of comparable concentration to residues in human blood. In men with a sperm count < 20 million/mL, there was a significant inverse relationship between sperm motility and the concentration of PCBs 153, 138, and 118, three congeners that are found in a large proportion of the general population (Bush et al. 1984).

In a recently published study, Dallinga et al. (2002) explored the relationship between human semen quality and organochlorine compounds in blood and semen. Among 65 men selected from couples visiting the Maastricht University Hospital, The Netherlands, they selected 31 men on the basis of normal semen quality [progressively motile sperm concentration (PMSC) ≥ 10^7/mL], and 34 men who were selected based on PMSC ≤ 10^6/mL. Blood samples were analyzed for PCBs 118, 138, 153, and 180 and their metabolites. They found a weak positive relationship between individual and combined PCB concentrations and sperm morphology (for combined PCBs, $n = 36$, $R^2 = 0.15$, $p = 0.02$). Within a stratified analysis, they found negative correlations between combined PCB metabolites in blood and sperm count and PMSC.

In another recently published study of 305 Swedish men 18–21 years old from the general population, Richthoff et al. (2003) found weak but statistically significant negative associations between PCB-153 and both the testosterone: sex-hormone–binding globulin ratio and CASA-measured sperm motility. They did not find associations with other semen parameters. They did not analyze serum for other PCB congeners. In the present study, the highest PCB-153 tertile, compared with the lowest tertile, was inversely, although not significantly, associated with sperm concentration, motility, and morphology.

Although the present study was a cross-sectional study in which semen and blood samples were collected on the same day, the long biologic half-life for PCBs and *p,p′*-DDE (Brown 1994; Phillips et al. 1989b) and the relatively short time interval for spermatogenesis (3 months) make this limitation of less concern. When conducting a study within a "special population," such as an infertility clinic, there are concerns with the generalizability of the results to the general population. Although the men in the present study may not be representative of men in Massachusetts, generalizability may not necessarily be limited. It is a misconception that generalization from a study group depends on the study group's being a representative subgroup of the target population (Rothman and Greenland 1998). For generalizability to be limited, the men who visit the infertility clinic would need to differ from men in the general population at some level that alters their response to PCBs. Although it is possible that men who visit this infertility clinic may differ from men in the general population and therefore be more "susceptible" to PCBs, this does not limit the internal validity of the study. On the contrary, the hypothesized increased susceptibility among infertility clinic men is an advantage of the design of the study because the most efficient way to explore whether environmental compounds affect human semen parameters is to target susceptible study populations. This design principle is practiced in other areas of epidemiology, such as when we study children, the elderly, or chronically ill individuals. It is efficient to target a study population that may be more susceptible to the exposure of interest because the study will be more powerful.

It is estimated that approximately 10–15% of couples in the United States are infertile (Speroff et al. 1999). Among the men in an infertile partnership, some are infertile or subfertile, whereas others are fertile. Therefore, the group of men presenting to the infertility clinic represents both men with reduced fertility and fertile men in infertile partnerships. Although the heterogeneity of the infertility clinic patients may increase the generalizability of the study results, the ultimate determination of generalizability awaits replication of this study in different populations.

In general, semen studies are challenging to conduct because participation rates are low (Bonde et al. 1996). General-population semen studies are the most challenging and may have very low participation rates, making it difficult to define the distribution of semen parameters in men from the general population. One subsample of the general population that has been studied are men attempting to conceive, believed to be more representative of men from

Table 7. Crude and adjusted ORs (95% CI) for below reference value sperm motility (SM)[a] by tertiles of grouped PCBs.

	Tertiles of PCB congener groupings			*p*-Value
	Tertile 1	Tertile 2	Tertile 3	for trend
ΣEstrogenic PCBs (group 1)				
SM < 50% motile (*n*)	26	34	37	
Crude OR (95% CI)	1.00	1.52 (0.75–3.06)	1.65 (0.83–3.31)	0.2
Adjusted OR (95% CI)[b]	1.00	1.40 (0.68–2.87)	1.41 (0.67–2.96)	0.4
ΣDioxin-like PCBs (group 2)				
SM < 50% motile (*n*)	28	31	38	
Crude OR (95% CI)	1.00	1.11 (0.55–2.22)	1.70 (0.85–3.41)	0.1
Adjusted OR (95% CI)[b]	1.00	0.97 (0.47–1.99)	1.49 (0.72–3.11)	0.3
ΣEnzyme-inducing PCBs (group 3)				
SM < 50% motile (*n*)	25	34	38	
Crude OR (95% CI)	1.00	1.71 (0.85–3.44)	2.12 (1.05–4.27)	0.04
Adjusted OR (95% CI)[b]	1.00	1.56 (0.76–3.21)	1.80 (0.84–3.88)	0.1

Tertile cut points (ng/g lipid): ΣEstrogenic PCBs: 3.9–12.5, 12.6–19.4, 19.5–204.8; ΣDioxin-like PCBs: 19.6–61.0, 61.4–94.1, 94.9–518.5; ΣEnzyme-inducing PCBs: 19.7–68.6, 69.7–111.1, 114.5–829.6.
[a]Below reference value for motility (SM) was defined as < 50% motile. [b]Adjusted for age (continuous), abstinence time (five categories: ≤ 2, 3, 4, 5, and ≥ 6 days) and smoking (current, former, and never).

Table 8. Crude and adjusted ORs (95% CI) for below reference value sperm morphology (SMPH)[a] by tertiles of grouped PCBs.

	Tertiles of PCB congener groupings			*p*-Value
	Tertile 1	Tertile 2	Tertile 3	for trend
ΣEstrogenic PCBs (group 1)				
SMPH < 4% normal (*n*)	17	19	22	
Crude OR (95% CI)	1.00	1.30 (0.58–2.92)	1.50 (0.68–3.33)	0.3
Adjusted OR (95% CI)[b]	1.00	1.11 (0.47–2.61)	1.47 (0.62–3.47)	0.4
ΣDioxin-like PCBs (group 2)				
SMPH < 4% normal (*n*)	16	18	24	
Crude OR (95% CI)	1.00	1.13 (0.50–2.56)	1.88 (0.84–4.19)	0.1
Adjusted OR (95% CI)[b]	1.00	1.01 (0.43–2.38)	1.68 (0.72–3.93)	0.2
ΣEnzyme-inducing PCBs (group 3)				
SMPH < 4% normal (*n*)	15	19	24	
Crude OR (95% CI)	1.00	1.59 (0.70–3.64)	2.23 (0.99–5.00)	0.05
Adjusted OR (95% CI)[b]	1.00	1.33 (0.56–3.16)	1.93 (0.79–4.72)	0.1

Tertile cut points (ng/g lipid): ΣEstrogenic PCBs: 3.9–12.5, 12.6–19.4, 19.5–204.8; ΣDioxin-like PCBs: 19.6–61.0, 61.4–94.1, 94.9–518.5; ΣEnzyme-inducing PCBs: 19.7–68.6, 69.7–111.1, 114.5–829.6.
[a]Below reference value for morphology (SMPH) was defined as < 4% normally shaped sperm. [b]Adjusted for age (continuous), abstinence time (five categories: ≤ 2, 3, 4, 5, and ≥ 6 days) and smoking (current, former, and never).

the general population than infertility clinic patients. In comparison with two recent well-conducted studies on men attempting to conceive, the percentage of men in our study with sperm concentration below the WHO reference value (18.8%) was higher than in Finnish men (4.5%) but similar to that found in Danish men (17.1% and 18.4%) (Bonde et al. 1998; Jensen et al. 2000). In a study on an unselected population, 25% of men 18–22 years old who participated in a compulsory examination for military service had sperm concentrations below the reference value (Andersen et al. 2000). Comparisons across studies and across countries are difficult because of differences in semen analysis techniques and because the role of geography on semen parameters remains unclear.

Conclusion

There was evidence of an inverse dose–response relationship between PCB-138 and sperm concentration, motility, and morphology. There was limited evidence of an inverse relationship of ΣPCBs and group 3 PCBs (cytochrome P450 enzyme inducers) with sperm motility and sperm morphology, as well as limited evidence of an inverse association between *p,p′*-DDE and sperm motility. The lack of a consistent relationship among semen parameters and other individual PCB congeners and congener groups 1 and 2 may indicate a difference in spermatotoxicity between congeners. Conversely, the associations found between semen parameters and PCB-138, ΣPCBs, and group 3 PCBs may be a reflection of conducting multiple comparisons. Data collection is ongoing in the present study, and these analyses will be rerun on a larger, more powerful data set. The results of the present study emphasize the need for a better understanding of the relationship between environmental chemicals and semen quality.

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