Published in final edited form as:

Reprod Toxicol. 2012 November ; 34(3): 429–435. doi:10.1016/j.reprotox.2012.04.006.

SERUM CONCENTRATIONS OF *p*, *p'*-DDE, HCB, PCBS AND REPRODUCTIVE HORMONES AMONG MEN OF REPRODUCTIVE AGE

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1. Background

Polychlorinated biphenyls (PCBs) were used extensively in the US until 1977 as insulators and lubricants in manufacturing [1]. Despite their discontinued use, they remain persistent in the environment because they are slow to break down in soil and bioaccumulate in fatty animal products in the food chain. Hexachlorobenzene (HCB) and dichlorodiphenyltrichloroethane (DDT), also no longer in use in the US by 1980, similarly continue to pose an exposure risk to humans [23]. Data from the National Health and Nutrition Examination Survey (NHANES) collected between 2003 and 2004 showed that PCB and numerous other persistent organic pollutants (POPs) were detectable in greater than 99% of the US population [4].

Several human studies have indicated an association between POPs and alterations in thyroid hormone levels, particularly inverse relationships with T3 and T4 [5]. There is also some evidence suggesting an effect on steroid hormones, though it is more limited [6-20]. *In vitro* and animal studies in males have shown associations between PCB exposure and decreased testosterone production and decreased conversion of progesterone to testosterone, though results have been inconsistent and differ by PCB congener [6-10]. In a study of adult male rats, the major DDT metabolite, dichlorodiphenyldichloroethylene (*p*, *p'*-DDE), was shown to be a strong androgen receptor antagonist [11], though there is some conflicting evidence [12]. Lastly, in an *in vitro* study of HCB, high levels were shown to interfere with androgen action, and low levels to enhance it [13].

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Associations between POP exposure and potentially downstream reproductive effects further suggest a relationship with steroid hormones. In Rhesus monkeys, PCB exposure was associated with decreased testicular size and changes in sperm morphology [6]. Changes in sperm morphology, motility, concentration and DNA integrity have similarly been observed in human studies of PCB exposure [14].

Human studies have also shown some suggestive relationships between POPs and altered reproductive hormone levels. Primarily, research has shown an association between PCB exposure and decreased testosterone, steroid hormone binding globulin (SHBG)-bound testosterone and SHBG to testosterone ratio [15-18]. Also, Asawasinsopon et al. [19] observed a significant inverse association between plasma levels of *p, p'*-DDE and estradiol levels. However, there have been inconsistencies in results across studies as well as in the exposure levels experienced by the populations studied. In this analysis we examined associations between several different PCB congeners, *p, p'*-DDE, and HCB with a panel of reproductive hormones in a population of US males (N=318) with exposure levels similar to those found among the US general population [20].

2. Material and methods

2.1 Study population

Subjects were male partners, aged 18-51, in subfertile couples seeking infertility evaluation and treatment at Massachusetts General Hospital (MGH) between January 2000 and May 2003 [21]. Males who were undergoing postvasectomy semen analysis or who were being treated for infertility with exogenous hormones were ineligible for participation [22]. Sixty-five percent of males (N=358) approached agreed to participate, and those who did not primarily reported lack of time on day of visit as their reason for refusal. IRB approval was received from MGH, Harvard and the University of Michigan.

2.2 PCBs, HCB and *p, p'*-DDE

Exposure measurements performed for this study were described in detail elsewhere [22]. Briefly, blood samples were collected from subjects between 9 a.m. and 4 p.m. on the day of visit, were centrifuged and the serum stored at -80 degrees C until analysis. Measurements were performed by the Organic Chemistry Analytical Laboratory at the Harvard School of Public Health. Gas chromatography with electron capture detection (GC/ECD) was performed to assess levels of POPs in our study, including 57 PCB congeners as well as *p, p'*-DDE and HCB in serum. Serum lipids were measured gravimetrically by weighing an aliquot of serum extract evaporated to dryness. Measurements (g/g serum) were included in full models as PCB levels are highly correlated with lipid concentration because they are lipophilic and accumulate in fatty tissues and serum lipid components, such as triglycerides [23]. Method detection limits (MDLs) for HCB and all PCB congeners were below 0.05 ng/g serum, with most congeners below 0.01 ng/g. The MDL for *p, p'*-DDE was the higher at 0.5 ng/g [21]. Individual congeners analyzed in this study were detectable in all subjects, and for grouping measures values below the MDLs were replaced with zeroes. Quality assurance and control (QA/QC) procedures were strictly followed by the Organic Chemistry Analytical Laboratory. These included participation in intercalibration exercises, such as the 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 organized by the Quebec National Institute of Public Health, Canada (three times per year) [22]. More detailed information on sampling, analytical and QA/QC procedures is described elsewhere [24].

PCB congeners examined in this analysis included 118, 138, 153 and 180, as well as a summed measure of all congeners and three groupings described by Wolff et al. [25]. Group 1 included potentially estrogenic and weak Phenobarbital inducers (congeners 44, 49, 52, 101, 187, 174, 177, 157/201); Group 2 included potentially antiestrogenic and dioxin-like compounds (congeners 95/66, 74, 77/110, 105/141, 118, 156, 167, 128, 138, 170); and Group 3 included Phenobarbital, CYP1A and CYP2B inducers (congeners 99, 153, 180, 196/203, 183).

2.3 Steroid hormones

Serum reproductive hormones were analyzed by the Reproductive Endocrine Unit (REU) Laboratory at MGH. Analytical methods and quality control procedures are described in detail elsewhere [26]. Hormones measured included follicle-stimulating hormone (FSH), luteinizing hormone (LH), inhibin B, total testosterone and estradiol (E_2), as well as sex hormone-binding globulin (SHBG). In addition, we explored associations with several ratio measures: total testosterone: SHBG to represent the free androgen index (FAI); total testosterone:LH (T:LH) as a marker of Leydig cell function; and total testosterone: E_2 (T: E_2) as a potential measure of aromatase activity. Finally, free testosterone (FT) concentration was calculated from a method described previously [27]. Most measurements for total testosterone, LH, FSH and SHBG were within reference ranges used by MGH. For total testosterone 52 subjects had levels less than 280 ng/dL and 1 subject had levels higher than 1,000 ng/dL. For LH zero subjects had levels below 1.6 IU/L and 10 subjects had levels above 25 IU/L. For FSH there were also zero subjects with measurements below 1.6 IU/L, but 51 above 12.5 IU/L. Lastly, for SHBG, there were 22 subjects with levels below 13 nmol/L and 1 subject with a level above 71 nmol/L. Small sample sizes prevented analysis within these subgroups.

2.4 Statistical analysis

Analysis was performed using R version 2.12.1 with the packages *epicalc* and *ggplot2*. We performed descriptive analyses to examine distributions of steroid hormones as well as lipid-standardized PCB congeners, HCB and *p,p'*-DDE. All exposure variables, as well as FSH, LH, SHBG, FAI and the T: E_2 ratio had a skewed distribution and were ln-transformed for analysis. Bivariate analyses using T-tests and one-way ANOVA were used to assess significant differences in exposure and outcome variables by the categorical covariates race/ethnicity, smoking status, time of blood draw, season of visit and year of visit. Spearman correlations were used to assess crude associations between the continuous covariates age and body mass index (BMI) and between individual exposures. Covariates that were significantly ($\alpha=0.05$) associated with exposure and/or outcome variables by one of these tests were considered for inclusion in multivariate models.

Crude and full regression models were created to assess relationships between POPs and reproductive hormones and ratio measures. Crude models included one wet weight POP concentration (ng/g serum), unadjusted for serum lipid content, and one hormone measure or ratio. Full models similarly included concentration of one wet weight POP and one outcome as well as serum lipid content, since using lipid-standardized POP values (i.e., measurements given in ng/g lipid) could unnecessarily cause increased measurement variability [28], although we did examine models with standardized measurements for comparison. Additional covariates in full models included those that fit the above criteria, meaning they were significantly associated with exposure and/or outcome variables, and additionally caused a change of greater than 10% in effect estimates upon inclusion in full models. All final models included the same covariates for consistency.

In secondary analyses we examined the relationships between quintiles of POP exposure with reproductive hormones to investigate possible non-linear relationships. These were performed for POP-hormone pairs that showed significant or near-significant relationships in both crude and adjusted models.

3. Results

3.1 Population characteristics

Of the 358 subjects enrolled in the study, 2 did not have hormone data and 15 were currently using hormonal medications and were excluded from analysis, leaving a final sample size of 341. Most participants were white (84%) and had never smoked (72%), and the average age and BMI were 36 and 28, respectively. Many participants (44%) showed no abnormalities in sperm concentration, motility or morphology, and a small proportion showed abnormalities in all three (11%). Relationships between semen quality characteristics and *p*, *p'*-DDE, HCB and PCB exposure were reported on a subset of this population by Hauser et al. [22]. Table 1 shows distributions of serum levels of lipid-standardized *p*, *p'*-DDE, HCB and PCB congeners examined in this analysis, as well as comparable measures that we calculated from data available in the National Health and Nutrition Examination Survey (NHANES) of males ages 18-51 between 2001 and 2004. The levels observed in our population were slightly higher than those in NHANES for all PCB congeners and HCB, and slightly lower for *p*, *p'*-DDE. Table 2 shows means and selected percentiles for serum reproductive hormones and ratios.

3.2 Bivariate relationships

In t-test and ANOVA analyses of mean differences by categorical covariates there were significant differences in *p*, *p'*-DDE exposure by race/ethnicity and smoking status, with higher concentrations in African Americans, Hispanics and other race/ethnicity compared to whites, and higher concentrations in ever smokers compared to never smokers. HCB levels were significantly lower in samples collected in the winter compared to other seasons. For PCBs, the only differences observed were for levels of PCB 180, which were lower in Hispanics compared to whites, and for Group 1 PCBs, which were higher in other race/ethnicity compared to whites. No other exposure variable showed differences by race/ethnicity, smoking status, season, time of day of sample collection or year of sample collection. For outcome measures, there were no significant differences by race/ethnicity. Current or ever smokers had lower FAI and FT levels compared to never smokers. Samples collected in the afternoon (1pm to 4pm) had significantly lower levels of inhibin B, total testosterone, FAI, T:E₂ ratio and FT compared to samples collected in the morning (9am to 12:59pm). Finally, inhibin B levels were higher in 2002 and 2003 compared to 2001, FAI levels were lower in all years compared to 2001, and E₂ levels were lower in 2002 and 2003 compared to 2001. Because of the inconsistencies in these observations, and because there did not appear to be any variables that were significantly associated with both exposure and outcome, none of these categorical covariates were considered for inclusion in full models.

Spearman correlations showed several significant relationships between POPs, reproductive hormones and continuous categorical covariates. Age and BMI were significantly associated with most exposure and outcome variables, and also caused greater than 10% changes in effect estimates when added into most regression models, and hence were included in all multivariate models. Additionally there were several significant correlations between exposure variables, similar to those observed previously [22,28]. Summed PCBs were most highly correlated with the individual congeners PCB 153 ($r=0.97$, $p<0.0001$) and PCB 138 ($r=0.95$, $p<0.0001$), and were highly correlated with all three groups (r for Group 1= 0.93 , r for Group 2= 0.95 , r for Group 3= 0.98 , p for all values <0.0001). Among individual congeners

PCBs 138 and 153 ($r=0.95$, $p<0.0001$) and PCBs 153 and 180 ($r=0.89$, $p<0.0001$) were most highly correlated. p , p' -DDE showed low to moderate correlation with HCB and PCB congeners and groups ($r<0.50$, $p<0.0001$), and HCB showed slightly stronger but still only moderate correlations with PCB congeners and groups ($r<0.60$, $p<0.0001$).

3.3 Crude and full model regression results

In crude regression models there were significant negative relationships of HCB and PCBs with SHBG, total testosterone, testosterone adjusted for SHBG, E_2 , T: E_2 ratio, T:LH ratio and FT (supplemental Table 1). Multivariate models, adjusted for serum lipids, age and BMI, are presented in Table 3. Adding these covariates to the models resulted in attenuation of most effect estimates. However, a significant negative relationship remained between PCB 118 and SHBG ($\beta=-0.13$, $p<0.01$), where an interquartile range (IQR) increase in PCB 118 corresponded to a 12.2% decrease (95% CI: -18.1%, -5.82%) in SHBG. SHBG was also suggestively inversely associated with Group 2 PCBs ($\beta=-0.08$, $p=0.08$), an IQR increase in Group 2 PCBs corresponded to a 6.01% decrease (95% CI: -12.34%, 0.8%) in SHBG. Total testosterone remained suggestively and inversely associated with PCB 118 ($\beta=-22.1$, $p=0.08$) and Group 2 PCBs ($\beta=-25.9$, $p=0.09$). No significant associations were observed for the hormones FSH, LH or inhibin B, either in crude or full models, though there were several suggestive relationships between FSH and PCBs 138, 153, 180 and Group 3 PCBs in crude models ($p<0.10$) (results presented in supplemental Tables 2 and 3). We additionally examined models using lipid-standardized POP values and found very similar results but with further attenuation of effect estimates (i.e., β closer to null) (results not shown). This is consistent with previous reports, which suggest that differences could be due to possible increased measurement error associated with this method of lipid adjustment [21,29].

3.4 Sensitivity analyses

Lastly we examined associations between POP exposure quintiles and hormone levels to examine non-linear relationships. From the results described previously we chose to focus on PCB 118 and Group 2 PCB concentrations in relation to SHBG, total testosterone and T:LH ratio (supplemental Table 4). The crude associations, with models unadjusted for lipids, showed significant decreasing monotonic trends in all models ($p<0.01$). Among models adjusted for serum lipids, age, and BMI, there was a significant but non-monotonic inverse trend between PCB 118 and SHBG (quintile increase in PCB 118 associated with 5.8% decrease in SHBG, $p<0.0001$) (Figure 1a). There was also a suggestive negative association between quintiles of PCB 118 and total testosterone (quintile increase in PCB 118 associated with 11.2 ng/dL decrease in testosterone level, $p=0.06$), though the relationship was not linear (Figure 1b); there was a monotonic decrease in total testosterone levels from PCB 118 quintiles 2 through 4, with higher testosterone levels, closer to the comparison group, for quintile 5. Only the 4th quintile was significantly different from the comparison group ($\beta=-50.0$, $p<0.01$). In adjusted models of Group 2 PCB quintiles, trends did not reach statistical significance but showed a suggestive decrease in SHBG and total testosterone for the highest exposure quintile (Figures 1c and 1d).

4. Discussion

Though several studies examined association between exposure to PCBs, p , p' -DDE and HCB and reproductive hormones in humans, none have thoroughly investigated relationships with such a large panel of exposures in populations of adult males of reproductive age exposed to levels on the order of those observed in the general US population. Studies of PCB associations with reproductive hormones have been primarily in groups highly exposed through fish consumption, and have varied greatly both by congeners measured and results. Goncharov et al. [18] observed an inverse relationship with PCB 153,

as well as the congeners 118, 138, 180 and a summed measure, with total testosterone. This population of Native Americans residing along the St. Lawrence River had high exposure levels (mean for PCB congener 153=123.7 ng/g lipid, as compared to 51.7 ng/g lipid in our population). Richtoff et al. [16] reported a significant inverse association between FAI and PCB 153, the only congener measured. The exposure levels in this group were closer in magnitude to ours (median for PCB 153=65 ng/g lipid), but the population was males ages 18-21 years. Summed PCB measures have been associated inversely with SHBG-bound testosterone levels [17] and positively with testosterone, FT, estradiol and aromatase index [30]. To our knowledge the latter study is the only one to observe positive relationships between steroid hormones and PCBs, and in a group with relatively low PCB levels (median for PCB congener 118=7.3 ng/g lipid), but the population was restricted to males ages 14-15 and effects may be differ from those observed in studies of older individuals. Lastly, Hagmar et al. [31] observed null associations between PCB 153 and a summed PCB measure and FSH, LH and free testosterone, at higher exposure levels than those in the present study (median for PCB 153=328 ng/g lipid).

In our study, after adjusting for serum lipids, age and BMI, we observed suggestive negative associations between PCB 118 and Group 2 PCBs with SHBG and total testosterone levels. These results are in some ways inconsistent with previous studies. No previous research has indicated an association between PCB 118 or summed Group 2 PCBs and altered testosterone levels. However, few studies have examined the relationships between these congeners specifically and serum sex hormones. Also, there is some evidence to suggest that these associations may have biologic plausibility. First, as mentioned previously, several *in vitro* and animal studies have demonstrated that some PCB congeners, though not PCB 118 and Group 2 PCBs specifically, cause a decrease in production of testosterone and of conversion of progesterone to testosterone [6-10]. Second, Group 2 PCBs and PCB 118 in particular are similar in structure to dioxins, which have been associated with decreased testosterone levels in humans [32-33]. Thus, though it is possible that these relationships are due to chance, they should be investigated more closely in toxicologic research.

Many of the previous studies of PCBs have also examined relationships between reproductive hormones and *p, p'*-DDE, again primarily at levels much higher than those observed in the general US population. Blanco-Muñoz et al. [34] observed an inverse relationship with testosterone and a positive relationship with inhibin B in a population of Mexican flower growers (median=677.2 ng/g lipid in rainy season, 626.7 ng/g lipid in dry season). An inverse association has been observed between *p, p'*-DDE and estradiol in two studies (median *p, p'*-DDE= 4057.7 ng/g lipid and 580 ng/g lipid) [19,35], but this finding is contrary to results from Dhooze et al. [30] where a positive relationship was observed among male adolescents with lower exposure levels (median *p, p'*-DDE=103.6 ng/g lipid). Lastly, Giwercman et al. [36] observed a significant positive relationship between *p, p'*-DDE and FSH in a pooled population of Swedish fisherman and men from Greenland, Poland and Ukraine (median=530 ng/g lipid). Our findings are inconsistent with these results, as we did not observe relationships between *p, p'*-DDE and any reproductive hormones in crude or full models. However, the literature is not conclusive of an effect, as other studies have also reported null associations [18,31,36-37]. HCB has also been examined along with these POPs in most studies, but results have been largely insignificant, which is consistent with our results [18,31,37].

A general inconsistency between our findings and many of the previous reports is a rather broad absence of association between the POPs measured and reproductive hormones. We did not observe, for example, an inverse relationship between testosterone and PCB 153 and summed PCBs as reported by Goncharov et al. [18]. Likewise, we did not observe inverse relationships between PCBs and SHBG-adjusted testosterone, or FAI index, as reported by

the Great Lakes Consortium [15,17]. These differences, as mentioned previously, could be due to a difference in exposure levels, as those observed in our subjects are lower than most previous studies and more representative of those observed in the general US population. Our null findings could be indicative of a threshold of exposure necessary for impacts on circulating hormone levels. On the other hand, our observation of a lack of association between POPs and the hormones FSH, LH and inhibin B were consistent with several other groups representing a range of exposure levels [15-16,31].

We observed stronger negative associations, primarily in models for testosterone, SHBG and several of the ratio measures, in crude models without lipid adjustment. Because POPs are lipophilic and accumulate in fatty tissues, adjustment for this covariate is necessary. However, the relationship between reproductive hormones and serum lipid content is less clear. In our dataset there were no significant Spearman correlations ($p < 0.05$) between serum lipids and FSH, LH or FAI, though there were significant correlations with the remaining hormones and ratio measures. There is some evidence that suppressed androgen levels are related to altered lipid profile [38-40]. The data suggest that this relationship may be causal, where low androgen levels lead to an altered balance of blood fat levels, for example, causing an increase in high-density lipoproteins (HDLs) and overall cholesterol levels [40]. This indicates that we may have observed reverse causation in our crude analysis, where changes in reproductive hormone levels are leading to altered lipid profiles and consequently changes in measurable levels of POPs.

There were several limitations in our study. First, only one serum sample was taken for both hormone and exposure measurements. However, several studies have shown that there is little temporal variability in most reproductive hormone measurements [41-44], except testosterone [43]. Furthermore, because POPs have, by definition, long half-lives, individual levels are unlikely to vary greatly over time. A second potential limitation is the use of a study population of males presenting to an infertility clinic, as it remains unclear whether our results are generalizable. However, men in the present study did have POP exposure levels similar to males in the general population, and included men with reproductive hormone levels within the normal range. Thus, they would need to respond differentially to PCBs compared to men not attending infertility clinics for our results to not be generalizable. Also this population lacked information on potential covariates that might have described exposure routes, such as freshwater fish consumption. A final limitation to our study was that we examined a large array of comparisons without using procedures to protect against Type I error. Therefore it is possible that our significant findings are due to chance.

5. Conclusions

Despite these limitations our study provides notable results. It is the first study to investigate the relationship between a wide range of POP exposures, at levels comparable to those in the general US population, with sex hormones in men of reproductive age. We observed many significant relationships in crude models without lipid adjustment, however in full models with the inclusion of serum lipids, age and BMI as covariates nearly no associations between POPs and reproductive hormone levels were observed. Further assessment of the relationships between POPs and reproductive hormones is needed, with an eye toward mechanistic understanding of the role of serum lipids and the potential for an exposure threshold for effects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

PCBs	Polychlorinated biphenyls
HCB	hexachlorobenzene
<i>p, p'</i>-DDE	dichlorodiphenyldichloroethylene
DDT	dichlorodiphenyltrichloroethane
POPs	persistent organic pollutants
NHANES	National Health and Nutrition Examination Survey
GC/ECD	gas chromatography with electron capture detection
MDLs	method detection limits
BMI	body mass index
IQR	interquartile range

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Research highlights

- We examined relationships between PCB exposure and sex hormones in adult males with fertility problems.
- Exposure was at levels consistent with those observed in the general US population.
- In crude models, PCBs were negatively associated with several sex hormones.
- In full models results were attenuated but some significant relationships remained.
- PCB exposure may be associated with circulating sex hormone levels in adult males.

A

B

c

D

Figure 1.

Adjusted^a regression coefficients (95% CI) for change in reproductive hormone measures in relation to PCB quintiles for (a) PCB 118 and SHBG; (b) PCB 118 and total testosterone; (c) Group 2 PCBs^b and SHBG; and (d) Group 2 PCBs^b and total testosterone.

Table 1

Distribution of serum concentrations of *p,p'*-DDE, HCB and PCBs, adjusted for serum lipids (ng/g lipid; N=341).

	Geometric Mean (95% CI)	Geometric Mean (95% CI) NHANES 01-04 ^d	Selected Percentiles						
			5 th	10 th	25 th	50 th	75 th	90 th	95 th
<i>p,p'</i> -DDE	236 (216, 258)	243 (215, 275)	87.7	104	141	204	329	613	1230
HCB	15.6 (14.9, 16.3)	10.4 (10.1, 10.7)	8.52	9.44	11.7	14.9	20.1	27.4	35.5
PCB-118	12.3 (11.5, 13.2)	5.1 (4.69, 5.48)	4.37	5.79	7.66	12.2	18.6	28.0	33.3
PCB-138	33.2 (31.2, 35.4)	16.7 (14.6, 19.2)	14.3	17.0	22.2	31.2	47.1	74.6	91.6
PCB-153	42.6 (40.0, 45.3)	24.1 (21.2, 27.3)	18.1	22.3	27.8	40.4	60.4	91.2	122
PCB-180	28.6 (26.7, 30.6)	17.3 (15.4, 19.4)	10.4	13.2	18.3	28.3	42.1	60.6	83.3
Σ PCB	222 (210, 235)		98.1	122	153	212	308	436	566
Σ Estrogenic PCBs (Group 1) ^b	16.1 (15.1, 17.2)		6.83	7.91	10.6	15.7	22.4	33.4	45.0
Σ Dioxin-like PCBs (Group 2) ^c	80.6 (76.0, 85.5)		37.7	43.3	54.8	74.4	116	166	215
Σ Enzyme-inducing PCBs (Group 3) ^d	90.7 (85.3, 96.4)		37.1	47.0	60.0	87.8	129	181	257

^aData calculated with appropriate weightings in Males ages 18-51. Summed and grouped variables were not calculated because NHANES did not include measurements on all PCBs examined in our dataset.

^bIncludes PCB congeners 44, 49, 52, 101, 187, 174, 177 and 157/201.

^cIncludes PCB congeners 95/66, 74, 77/110, 105/141, 118, 156, 167, 128, 138 and 170.

^dIncludes PCB congeners 99, 153, 180, 196/203 and 183.

Table 2

Distribution of serum reproductive hormone levels and ratio measures (N=341).

Hormone	Reference ranges ^b	Geometric Mean	Selected Percentiles							
			5 th	10 th	25 th	50 th	75 th	90 th	95 th	
Follicle-stimulating hormone (IU/L)	1.6 – 12.5	7.82	3.65	4.30	5.53	7.33	10.3	15.1	22.7	
Luteinizing hormone (IU/L)	1.6 – 25	10.2	4.98	5.71	7.46	10.3	13.8	17.2	21.6	
Inhibin B (pg/mL) ^a		159	57.4	79.1	116	152	189	251	281	
Sex hormone-binding globulin (nmol/mL)	13 – 71	25.8	12.5	15.1	20.0	25.8	34.4	45.7	51.3	
Testosterone (ng/dL) ^a	280 - 1000	423	216	247	318	404	512	619	685	
Free testosterone (ng/dL) ^a		9.43	5.27	6.05	7.43	9.12	11.3	13.6	14.9	
Free androgen index		53.2	30.7	34.5	42.1	52.1	68.2	83.9	96.7	
Estradiol (pmol/L) ^a	20 - 77	31.9	10.0	20.0	25.0	31.0	38.0	45.0	50.0	
Testosterone: estradiol ratio		13.4	6.61	7.65	9.75	13.1	17.7	23.8	31.7	
Testosterone: luteinizing hormone ratio		38.8	16.1	21.3	30.4	40.1	54.0	70.8	79.8	

^a Arithmetic mean

^b Normal ranges from REU Laboratory at MGH. Reference range for Inhibin B and ratio measures not established.

Table 3

Adjusted^a regression coefficients (95% CI) for change in hormone level associated with an ln-unit change in POP concentration (N=341).

	SHBG ^b	Testosterone	Testosterone ^c	Free T	FAI ^b	E ₂	T:E ₂ ^b	T:LH ^b
p,p-DDE	-0.03 (-0.09, 0.02)	5.54 (-12.8, 23.9)	11.6 (-3.80, 27.1)	0.25 (-0.12, 0.62)	0.03 (-0.02, 0.08)	0.59 (-0.89, 2.08)	-0.03 (-0.09, 0.03)	-0.03 (-0.09, 0.04)
HCB	-0.06 (-0.17, 0.05)	-21.7 (-58.6, 15.1)	-10.7 (-41.8, 20.4)	-0.28 (-1.03, 0.47)	-0.01 (-0.10, 0.09)	-0.52 (-3.51, 2.47)	-0.07 (-0.20, 0.05)	0.03 (-0.10, 0.17)
PCB118	-0.13 (-0.20, -0.06) ***	-22.1 (-46.5, 2.36) *	1.75 (-19.3, 22.8)	0.12 (-0.38, 0.62)	0.08 (0.01, 0.14)	-0.84 (-2.83, 1.14)	-0.01 (-0.09, 0.07)	-0.02 (-0.11, 0.07)
PCB138	-0.33 (-0.11, 0.04)	-16.4 (-43.3, 10.5)	-10.2 (-32.8, 12.5)	-0.21 (-0.76, 0.33)	-0.003 (-0.08, 0.07)	-1.51 (-3.69, 0.66)	0.01 (-0.08, 0.10)	-0.06 (-0.15, 0.04)
PCB153	-0.03 (-0.11, 0.05)	-11.5 (-39.6, 16.7)	-6.12 (-29.8, 17.6)	-0.14 (-0.71, 0.43)	0.003 (-0.07, 0.08)	-1.13 (-3.41, 1.15)	0.02 (-0.08, 0.11)	-0.03 (-0.13, 0.07)
PCB180	0.02 (-0.06, 0.10)	0.61 (-27.0, 28.2)	-3.97 (-27.2, 19.2)	-0.14 (-0.70, 0.42)	-0.02 (-0.09, 0.05)	-0.75 (-2.98, 1.48)	0.02 (-0.07, 0.12)	0.01 (-0.09, 0.11)
ΣPCB	-0.05 (-0.14, 0.04)	-14.1 (-44.3, 16.1)	-5.07 (-30.6, 20.4)	-0.14 (-0.75, 0.48)	0.02 (-0.07, 0.10)	-1.20 (-3.65, 1.25)	0.005 (-0.10, 0.11)	-0.03 (-0.14, 0.08)
Group1	-0.05 (-0.13, 0.02)	-16.7 (-43.3, 9.91)	-6.46 (-29.0, 16.1)	-0.16 (-0.70, 0.38)	0.01 (-0.06, 0.08)	-0.94 (-3.10, 1.22)	-0.03 (-0.12, 0.06)	-0.03 (-0.13, 0.07)
Group2	-0.08 (-0.17, 0.01) *	-25.9 (-56.2, 4.42) *	-11.4 (-37.1, 14.3)	-0.30 (-0.92, 0.32)	0.01 (-0.07, 0.10)	-1.75 (-4.21, 0.70)	-0.004 (-0.10, 0.10)	-0.05 (-0.16, 0.06)
Group3	-0.01 (-0.10, 0.07)	-5.1 (-35.6, 25.4)	-2.65 (-28.3, 23.0)	-0.12 (-0.74, 0.50)	0.001 (-0.08, 0.08)	-1.08 (-3.55, 1.39)	0.02 (-0.08, 0.12)	-0.01 (-0.12, 0.10)

***p<0.05

^aAdjusted for age, BMI and serum lipids.

^bHormone variable was transformed using the natural log (ln).

^cTestosterone model adjusted for ln(SHBG) as a covariate.

* p<0.10

*** p<0.01