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A case-control study of polychlorinated biphenyl association with metabolic and hormonal outcomes in polycystic ovary syndrome

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Abstract

PCBs are a class of environmental pollutants with a long half-life that sequester in fat. Women with PCOS may represent a sensitive subgroup to endogenous exposure to PCBs because of associated weight gain. 7 PCB congeners were compared in age, ethnicity, and BMI matched women with (n=29) and without (n=30) PCOS and related to metabolic outcomes, and steroid and thyroid hormone levels. PCB118, PCB138, PCB153 and PCB180 were detected in all serum samples but geometric mean did not differ between cases and controls. PCBs correlated with increasing concentrations of each other ($p < 0.01$), increasing age ($p < 0.01$) and decreasing lneGFR ($p < 0.05$). lnPCB118 correlated with increasing Free-T4 ($p = 0.028$). lnPCB158, lnPCB180 and ln∑PCB correlated with increasing lnSHBG ($p < 0.044$). In regression modelling, although not significant, PCB118 positively associated with lnSHBG in controls ($p = 0.0504$) but not in cases; estradiol inversely associated with PCB138 in controls ($p = 0.055$) and ∑PCB in cases ($p = 0.051$). No significant associations were observed between metabolic endpoints, and steroid and thyroid hormone levels. The results presented do not suggest the PCOS cases in this cohort are at adverse risk compared to age, ethnicity, and BMI matched controls.

Key words: polycystic ovarian syndrome (PCOS), endocrine disrupters, polychlorinated biphenyls (PCBs)

Abbreviations: PCB(s), polychlorinated biphenyl(s); PCOS, polycystic ovarian syndrome; IVF, In Vitro Fertilization; BMI, body mass index; CRP, C reactive protein; eGFR, estimated glomerular filtration rate; SHBG, sex hormone binding globulin; HbA1c, glycosylated hemoglobin A1c; HOMA-IR, homeostatic model assessment for insulin resistance; FAI, free androgen index; TSH, thyroid stimulating hormone; Free-T4, free-thyroxine; Free-T3, free-triiodothyronine; TC, total cholesterol; TG, triglycerides; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; LC/MS/MS, liquid chromatography tandem mass spectrometry; CP0, coplanar non-ortho; CP1, coplanar mono-ortho; PP, both para positions (4, 4') chlorinated, 4CL, ≥ 4 chlorine atoms; 2M, ≥ 2 meta positions (3, 3', 5, and 5') chlorinated; LOR, limit of reporting. LOR, limit of reporting; DCM, dichloromethane; GM, geometric mean; β, regression coefficients; Cl, confidence interval; In, natural log transformation; SD, standard deviation; n, sample size; HRGC/HRMS, high resolution gas chromatography coupled with high resolution mass spectrometry.

1. Introduction

Polychlorinated biphenyls (PCBs), anthropogenic organic compounds with chlorine covalently bound to biphenyl, were widely manufactured from the 1920's for commercial use as electrical fluids, as additives in plasticizers and fire retardants, and in a variety of products including caulks, adhesives, plastics, and carbonless copy paper.[1] Although banned since the 1980's, highly chlorinated PCBs (≥ 5 chlorine atoms) are generally considered to be resistant to bio-transformations, are retained in adipose tissue or in plasma due to their high lipophilicity,[2] and persist in the environment. Dietary exposure is the main cause of PCB accumulation in humans, particularly from high fat-foods such as fish, meat, poultry and dairy products.[3] Elimination is primarily in feces, and to a lesser extent, in sweat and urine.[4] In breastfeeding mothers, lactation is a primary route of PCB removal.[5] Intrinsic elimination half-lives based on a UK population for PCB118, 138, 158 and 180 are estimated to be 9.3, 10.8, 14.4 and 11.5 years, respectively.[6]

PCBs have been associated with reproductive health outcomes in women including irregular menses,[7] fewer lifetime pregnancies,[8] endometriosis,[9] shorter fundi and uteri lengths,[10] fibroids,[11] polycystic ovarian syndrome (PCOS),[12,13] and primary ovarian insufficiency.[14] Furthermore, PCBs are associated with diabetes,[15,16] and thyroid function.[17-20] *In vitro,* PCB153 is reported to have antiestrogenic activity,[21] to preferentially accumulate in the wall of porcine follicles, exhibiting both estrogenic and antiestrogenic effects and to effect steroidogenesis.[22] PCB180 is reported to have antiestrogenic activity,[21] and to act as a thyroid receptor agonist *in vivo*.[23] *In vitro*, PCB138 demonstrated both antiestrogenic and antiandrogenic activity.[21] High concentrations of PCBs have also been linked to lowered estradiol levels.[10]

PCOS is the most common endocrine disorder among women of reproductive age with a reported prevalence of 6% - 10%,[24] and is associated with menstrual dysfunction, infertility,

hirsutism, acne, obesity, and metabolic syndrome.[25] Given previous reports of increased risk of PCOS in women exposed to PCBs,[12,13] PCOS women undergoing fertility treatment may be at greater adverse risk of the endocrine disrupting effects of these persistant organic pollutants. The aim of this study was to examine correlation of serum measurements of PCBs, and to explore the associations of PCBs with hormonal parameters in a homogenous cohort of women with and without PCOS who were age and weight matched.

2. Materials and Methods

2.1 Study design

This case-control study was performed within the Hull In Vitro Fertilization (IVF) Unit, UK, in 2015. Ethical approval was obtained by The Yorkshire and The Humber NRES ethical committee, UK (approval number 02/03/043) and all participants gave their written informed consent.[26] Participants were recruited sequentially with the exclusion of known immunological disease, diabetes, renal or liver insufficiency, acute or chronic infections, or inflammatory diseases, < 20 years, > 45 years, body mass index (BMI) > 35 and those not undergoing IVF treatment. PCOS was diagnosed using the revised 2003 Rotterdam criteria.[27] All women were Caucasian from the same geographical area in northern England within a 20-mile radius from the IVF center.

2.2 Sample collection

A fasting blood sample was taken on day 21 of the luteal phase of cycle, prior to commencement of IVF treatment. Blood samples were centrifuged at 3500 × g for 15 min at 4 °C and stored at −80°C within 1 h of collection until analysis. Plasma glucose was measured using a Synchron

LX20 analyzer (Beckman-Coulter). Serum insulin was assayed using a competitive chemiluminescent immunoassay performed using the DPC Immulite 2000 analyzer (Euro/DPC, Llanberis, UK). C reactive protein (CRP) was measured enzymatically using a Synchron LX20 analyzer (Beckman-Coulter, UK). Total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-c) were measured enzymatically using a Synchron LX20 analyzer (Beckman-Coulter, High Wycombe, UK). Low-density lipoprotein cholesterol (LDL-c) was calculated using the Friedewald equation.[28] Estradiol, thyroid stimulating hormone (TSH), free thyroxine (Free-T4) and free triiodothyronine (Free-T3) assays were performed on an Abbott Architect i4000 immunoassay analyzer (Abbott Diagnostics Division, UK). Serum testosterone and androstenedione were measured by liquid chromatography tandem mass spectrometry (LC/MS/MS; Acquity UPLC-Quattro Premier XE-MS, Waters, Manchester, UK). Sex hormone binding globulin (SHBG) was measured by an immunometric assay with fluorescence detection (DPC Immulite 2000 analyzer: upper limit 2.0 nmol/L). Glycosylated hemoglobin A1c (HbA1c) measurements were made using ion-exchange chromatography. Estimated glomerular filtration rate (eGFR mL/min/1.73 m^2) was calculated using the formula eGFR =175 x (SCr, mg/dL)$^{-1.154}$ x (age, years)$^{-0.203}$ x 0.742. Total serum lipids were calculated from TC and TG using the short formula (2.27 x TC) + TG + 62.3 mg/dL. Insulin resistance was calculated from basal glucose and insulin concentration using the homeostasis model assessment for insulin resistance (HOMA-IR) ((Insulin x glucose)/22.5). Free androgen index (FAI) was calculated from serum testosterone and SHBG concentrations using the formula ((testosterone/SHBG) x 100). A sum PCB (∑PCB) variable was calculated by adding the molar concentrations of the PCB congeners (i.e., sum of PCB28, PCB52, PCB101, PCB118, PCB138, PCB153, PCB180).

2.3 Extraction and clean-up

Extraction and clean-up of PCBs in serum samples was performed using a previously described protocol.[29] Briefly, 5 mL of serum was aliquoted into 50 mL Falcon tubes and spiked with 5 ng of each of $^{13}C_{12}$-labelled PCBs -28, -52, -101, -118, -138, -153 and -180 (Wellington Laboratories). Samples were vortexed for 1 minute and left to stand for 30 minutes. 6 mL acetonitrile, 3 mL milliQ, 5 g anhydrous MgSO4 and 1 g NaCl were added along with a ceramic homogenizer. Samples were manually shaken for 1 minute, before being centrifuged at 4500 RPM for 8 minutes at 10 °C. The supernatant layer was collected and transferred to a glass tube. The extract was evaporated to near-dryness on a hot plate using a gentle stream of nitrogen and reconstituted in approximately 1 mL hexane. 1 mL of >98% concentrated sulfuric acid was added, and the sample was vortexed for at least 30 s. The aqueous and organic layers were left to separate overnight at <4 °C. The supernatant (hexane) layer was transferred directly onto a silica SPE cartridge (Supelco LC-Si, 3 mL/500 mg) (preconditioned with 6 mL dichloromethane (DCM), followed by 6 mL hexane). Target compounds were eluted into a 15 mL glass collection tube using 6 mL hexane. Clean extracts were evaporated to near-dryness, reconstituted in 50 µL hexane containing 2.5 ng $^{13}C_{12}$-PCB-141 as a recovery standard, and transferred to inserted autosampler vials prior to analysis.

2.4 Instrumental analysis

PCBs were determined in serum samples by high resolution gas chromatography coupled with high resolution mass spectrometry (HRGC/HRMS) using previously described methods.[30] Briefly, a Thermofisher TRACE 1300 gas chromatograph was coupled to a Thermofisher DFS mass spectrometer. The injector was operated in splitless mode with separation achieved on an

Agilent DB-5ms column (30 m length x 0.25 mm in diameter x 0.25 μm film thickness). Experiments were conducted in MID mode at 10,000 resolution (10% valley definition). The inlet, transfer line and source were held at 250 °C, 280 °C and 280 °C, respectively. The flow rate was maintained at 1.0 mL/min. In the GC oven, an initial temperature of 80 °C was held for two minutes before ramping to 180 °C at 20 °C/min and held for 0.5 min. The temperature was then increased to 300 °C at 10 °C/min and held for 5 min.

2.5 Quality assurance/quality control

A blank sample was extracted as every 6th sample (n=10) alternating between 5 mL of MilliQ (reagent blank) and 5 mL bovine serum (field blank). If a target compound was detected in a blank at less than 5% of the measured sample concentration, then no blank correction occurred. No blank samples contained target compounds at concentrations >5% of samples concentrations. In the absence of a certified QC sample, method precision and accuracy were determined using bovine serum (5 mL, n=5) fortified with target compounds. 30 μL of solution containing 0.2 ng/μL of all target compounds in methanol was added to each aliquot, which was then vortexed for 1 min and left at <4 °C overnight. Samples were analyzed as real samples using the protocols described above. Good accuracy and precision were found for all target analytes with average recoveries between 80 and 120% and a relative standard deviation of <15%.

2.6 Data analysis and statistics

With no comparative study on which to base formal power calculations, a minimum of 20 degrees of freedom was used to estimate residual variance.[31] 25 participants per group, plus 5 additional to allow for dropout, were planned to be recruited. A total of 59 Caucasian women were recruited into the study, 29 PCOS cases and 30 control subjects, age and BMI matched.

Descriptive data are presented as mean ± SD for continuous data and n (%) for categorical data. Serum PCB concentrations are expressed as geometric mean with 95% confidence intervals. Measured serum PCB, hormone concentrations, and metabolic markers were assessed for normality, extreme outliers were removed and/or were ln transformed, as required. Independent T and Mann-Whitney U tests were used to compare means/medians, as appropriate and chi-square test of association for categorical variables. Potential associations were examined using Pearson's product moment correlations or Spearman's rank order correlations. A p-value of < 0.05 was considered to indicate statistical significance.

Multivariable linear regression modelling was carried out as an initial step to identify potential covariates. Age, smoking, BMI, eGFR, albumin and total serum lipids were considered as potential covariates and were retained in models for a given set of endpoints (steroid hormones, thyroid hormones, and metabolic outcomes) if they were significant at $p < 0.1$ or if they were influential on the serum PCB coefficient for at least one of the regressions. To assess the potential associations between measured steroid hormones, thyroid hormones, or metabolic outcomes and serum PCB concentrations, multivariable linear regression analysis stratified by PCOS case status was performed. Assumptions of linearity, homoscedasticity, absence of multicollinearity, and normality were met. To examine potential associations between FAI and PCBs, non-parametric quantile regression of the median was carried out due to non-normal distribution of the residuals. Tobit regressions were used to examine potential associations between estradiol concentrations and PCBs due to left censoring of the data (concentrations below 75 pmol/L were not quantified). A p-value of < 0.05 was considered to indicate statistical significance in regression analysis. Statistical analyses were conducted using SPSS (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp).

Tobit and quantile regressions were carried out using STATA (IC 16.1, Stata Corp., College Station, TX).

3. Results

7 PCB congeners were detected (Table 1). PCB118, PCB138, PCB153 and PCB180 were detected in all serum samples. The detection frequencies for PCB28, PCB52 and PCB101 were < 50%; 25%, 6.8% and 44%, respectively. PCB28 and PCB101 were detected in PCOS cases and controls. PCB52 was detected in PCOS cases but not controls. Of the most frequently detected PCBs, PCB153 was the most abundant followed by PCB138, PCB180 and PCB118. Geometric mean concentration of PCBs did not differ between PCOS cases and controls (Table 2).

[Insert Table 1. here]

[Insert Table 2. here]

PCOS cases were statistical significantly more likely to be non-smokers (0% versus 20%, p = 0.024) (Table 3). Age, BMI, insulin, fasting blood glucose, HbA1c, HOMA-IR did not differ between cases and controls. PCOS cases had higher levels of androgens compared to controls with statistically significant higher FAI (3.11 versus 1.71, p = 0.004), testosterone (1.13 versus 0.77 nmol/L, p = 0.006) and androstenedione (4.1 versus 2.53 nmol/L, p = 0.0004). SHBG was significantly lower in PCOS cases (64.63 versus 109.73 nmol/L, p = 0.042) and there was no difference between estradiol. PCOS cases had significantly lower eGFR (88.14 versus 97.38 mL/min/1.73 m^2, p = 0.037) but CRP did not differ between cases and controls. TSH, Free-T4 and Free-T3 did not differ between cases and controls. HDL-c was significantly higher in controls (1.65 versus 1.41 mmol/L, p = 0.010). Cholesterol, triglycerides, LDL-c, and Total

serum lipids did not differ between cases and controls. For PCOS cases and controls, there was no difference between serum concentration of the frequently detected PCBs and menstrual cycle (irregular versus regular) (data not shown).

[Insert Table 3. here]

Pearson's product moment or Spearman's rank order correlations for the ln transformed concentrations of the frequently detected PCBs; InPCB118, InPCB138, InPCB153, InPCB180, ln∑PCB, were examined with age, lneGFR, serum albumin, BM1, total serum lipids, as well as metabolic, steroid, and thyroid hormone outcomes (Supplementary Tables S1 – S5). All lnPCBs correlated with increasing concentrations of each other ($r > 0.7$, $p < 0.01$), increasing age ($r > 0.40$, $p < 0.01$) and decreasing lneGFR ($r > -0.24$, $p < 0.05$). No correlations with BM1, albumin, or total serum lipids were observed for any of the PCBs. lnPCB158, lnPCB180 and ln∑PCB correlated with increasing lnSHBG ($r = 0.270$, $p = 0.044$; $r = 0.362$, $p = 0.006$; $r = 0.294$, $p = 0.028$, respectively). lnPCB180 and ln∑PCB correlated with decreasing lnFAI ($r = -0.298$, $p = 0.029$; $r = -273$, $p = 0.046$). No other correlations with PCBs were observed for steroid hormone outcomes. lnPCB118 correlated with increasing Free-T4 ($\rho = 0.311$, $p = 0.028$). No correlations with TSH and Free-T3 were observed. No correlations with metabolic outcomes, fasting blood glucose, insulin, HOMA-IR or HbA1c were observed.

Unstratified multivariable linear regression analysis for covariate selection, with metabolic and hormone outcomes as dependent variables, and frequently detected PCBs and ∑PCBs and potential covariates (age, smoking, BMI, eGFR, albumin, total serum lipids and metformin use) as independent variables, yielded a positive association between HbA1c and PCB118 (0.73 mmol/mol increase per ng/mg Lipid increase of PCB118, $p = 0.049$). Estradiol

was negatively associated with PCB138, PCB153 and ∑PCBs (-25.84 pmol/L (p = 0.040), -17.44 pmol/L (p = 0.040) and -7.82 pmol/L (p = 0.030) per ng/mg Lipid increase of PCB138, PCB153 and ∑PCBs, respectively). No other associations between metabolic outcomes, and steroid and hormone levels, and serum PCB or ∑PCB concentrations were identified (data not shown).

Associations between metabolic endpoints and serum PCBs, stratified by PCOS case status, were assessed in multivariable linear regression considering potential covariates (Table 4). Of the potential covariates, only BMI and age were included in final models. No associations were observed in PCOS cases or controls between fasting blood glucose, lnHOMA-IR, lnInsulin or HbA1c, and serum PCB or ∑PCB concentrations.

[Insert Table 4. here]

We examined associations between steroid hormone concentrations, stratified by PCOS case status, and serum PCB and ∑PCB concentrations adjusted for age, serum albumin and BMI (Table 5). There was no association in PCOS cases or controls between FAI, androstenedione and lntestosterone, and serum PCB or ∑PCB concentrations. Although not significant, PCB118 positively associated with lnSHBG in controls (0.197 ln increase per ng/mg Lipid increase of PCB118, p = 0.0504) but not in cases. Estradiol, although not significant, inversely associated with PCB138 in controls, and with the ∑PCB in cases (37.9 pmol/L (p = 0.055) and 9.7 pmol/L (p = 0.051) decrease per ng/mg Lipid increase in PCB138 and ∑PCB, respectively). No associations between steroid hormone concentrations and serum PCB and ∑PCB concentrations were observed.

[Insert Table 5. here]

There were no associations observed between the frequently detected PCBs or the ∑PCB with thyroid hormones concentrations measured, TSH, lnFree-T4 and lnFree-T3, adjusted for serum albumin and smoking (Table 6).

[Insert Table 6. here]

4. Discussion

Altered reproductive hormones, classically hyperandrogenism, is typical in PCOS. Given PCBs are reported to have altered endocrine activity effects,[32] and have been associated with increased risk of PCOS,[12,13] PCOS subjects may therefore be at greater adverse risk to exogenous exposure to these persistent organic pollutants. In this study, PCOS subjects were carefully matched in age, ethnicity, and BMI to the control subjects to ensure that any changes that may have been seen could be associated with PCOS. In addition, as these environmental pollutants are lipophilic, all the PCOS and controls were not obese that may have been a confounder in previous studies where often PCOS subjects are obese and of greater weight than controls. Notably as the PCOS subjects were not overweight in this study then they were not insulin resistant as normally seen in this population.

4.1 PCB detection

Although PCBs have been banned, PCBs congeners were detected in serum samples in participants in this study. Consistent with a longer intrinsic half-live, 14.4 years,[6] PCB153 was the most abundant PCB congener detected. The lower detection frequency of less chlorinated

PCB congeners detected in this study may be explained by their shorter intrinsic half-lives (PCB28 = 5.5 years, PCB52 = 2.6 years).[6] PCB118, PCB138, PCB153 and PCB180 were detected in all serum samples but geometric mean did not differ between PCOS cases and controls. Previous PCOS case control studies have mixed results. In Han women, Yang et al[12] found that PCOS cases had significantly elevated PCB118, PCB138, PCB153, PCB180 and ∑PCB in PCOS cases compared to controls. In a US population, Vagi et al[13] found that there was only a significant difference with PCB180 between PCOS cases and controls, with higher concentrations in controls versus cases. In this population, the researchers found that participants were 6-8 times more likely to have PCOS for whole weight (including PCB153 and PCB180) and lipid adjusted (including PCB180) PCB concentrations in the middle tertile when compared to the lowest tertile,[13] and in the Han cohort, significant associations were observed for individual PCB congeners, the sum of all PCBs, and all dioxin like PCBs with PCOS.[12] However, subjects were not weight and aged matched as in this study suggesting that PCBs are not linked to PCOS when these characteristics are considered.

Consistent with previous studies, frequently detected PCBs in this study all correlated with increasing concentrations of each other and with increasing age.[12,15] The concentration of frequently detected PCBs, however, were generally lower than that reported in other studies; Italy (PCB118 18.86 ng/g lipids, PCB138 63.86 ng/g lipids, PCB153 99.9 ng/g lipids, PCB180 124.1 ng/g lipids, n = 816, 32 – 66 years, 2013 – 2014);[33] America (PCB118 13.2 ng/g lipids, PCB138 39.8ng/g lipids, PCB153 39.8 ng/g lipids, PCB180 45.1 ng/g lipids, n = 412, 55 – 57 years, 2005 – 2007).[34]

4.2 Assessment of PCBs on metabolic endpoints

In the assessment of measured metabolic outcomes, fasting blood glucose, lnHOMA-IR, lnInsulin and HbA1c, frequently detected PCBs and ∑PCB were not found to correlate with metabolic outcomes, and no association between PCOS cases or controls between metabolic outcomes and serum PCB or ∑PCB concentrations were observed. The only notable association observed with metabolic outcomes was an increase in HbA1c associated with increasing serum PCB118 concentration (0.73 mmol/mol increase per ng/mg Lipid increase of PCB118, p = 0.049) in unstratified models with all potential covariates (data not shown).

PCBs have been linked to metabolic alterations related to diabetes in animal and epidemiological studies. In mice, PCB153 exposure resulted in glucose metabolic dysfunction,[35] and PCB138 and PCB118 led to induced insulin resistance.[36] In the CARLA and KORA cohorts in Germany, an increased association of incident diabetes was found for inter quartile range increase of PCB138 (OR: 1.50, 95% CI: 1.07 - 2.11) and PCB153 (OR: 1.53, 95% Cl: 1.15–2.04), with higher odds ratio in women.[15] Similar risk of diabetes was also found in middle aged women for the sum of dioxin like PCBs, including PCB118 (OR: 1.95, 95% Cl: 1.42 - 2.69).[16] Consistent with the results in this study, Yang et al[12] also found no difference in measured concentrations of fasting blood glucose, HOMA-IR or Insulin between PCOS cases and controls, and no correlation between the sum of PCBs fasting blood glucose, HOMA-IR or Insulin were found.

4.3 Assessment of PCBs on steroid hormone levels

Among steroid hormones measured, the only notable associations, although not significant, were a positive association between PCB118 and lnSHBG in controls, a negative association between

estradiol and PCB138 in controls but not PCOS, and a negative association between estradiol and the ∑PCB in PCOS but not controls. These negative associations with estradiol were observed to be significant in unstratified models when all potential covariates were considered. Further significant negative association was identified with PCB153 but was lost in stratified models (data not shown).

The association between the mono-ortho dioxin like PCB, PCB118, and SHBG in this study is consistent with that found in cord blood samples from mother-child pairs in a Japanese cohort.[37] The lack of association in PCOS cases may be a consequence of the relatively small number of PCOS cases (n = 27) with measured SHBG levels in addition to the significantly lower SHBG levels in cases compared to controls; 51.38 versus 109.73 nmol/L, p = 0.042. Conversely, there may be no association when PCOS are matched by weight and age with controls. The lower SHBG levels observed in PCOS cases is consistent with systematic review and meta-analysis of 39 studies where lower SHBG levels were associated with increased risk of PCOS (SMD: -0.83, 95% CI:-1.01, -0.64).[38] Although not associated, we did find that lnPCB153, lnPCB180 and ln∑PCB correlated with increasing lnSHBG, consistent with PCB and SHBG levels in cord blood samples in the PELAGIE birth cohort.[39] SHBG is known to affect the bioavailability of steroid hormones by preferential binding in the order of testosterone > androstenedione > estradiol with total testosterone represented by 1-2% circulating as free testosterone, 65% bound to SHBG and the remaining bound to albumin.[40] Given that SHBG concentrations influence testosterone concentrations, higher SHBG levels may have elevated total testosterone but low bioavailable and free testosterone concentrations,[40] indicating an indirect effect of PCBs on testosterone levels.

The consistent negative, although not significant association between estradiol, and PCB138 and the ∑PCB, in this study is similar to the results found by Tang et al[41] in umbilical cord blood in females. The researchers found that PCB congeners tested were consistently negatively associated with estradiol. In 8-year-old children exposed in utero, estradiol levels were found to be significantly lower in those exposed to higher levels of PCDD/Fs + PCBs compared to lower levels ($p = 0.003$).[10] Given the significant negative associations observed in unstratified models, the scattered results between cases and controls may be a result of the low number of measured estradiol levels in each group ($n = 18$ and $n = 17$, respectively) which resulted in left censoring of the data and subsequently Tobit regression analysis in stratified models.

4.4 Assessment of PCBs on thyroid hormone levels

In multivariable linear regression analysis with thyroid hormones, no associations were observed between TSH, Free-T4 and Free-T3, when adjusted for potential covariates. In this study, the only notable relationship between PCBs and thyroid hormone levels was the significant positive correlation of the coplanar dioxin like PCB, PCB118, and Free-T4 levels. No correlations between TSH and Free-T3 and PCB concentrations were observed.

In animal models, PCBs are associated with decreased total T4,[42] and Free-T4 levels.[23] However, there is a lack of consistent evidence in epidemiological studies. Takser et al[20] in a study of pregnant women reported a significant negative relationship between circulating total T3 at low environmental doses of PCB138, PCB153, PCB180 and found no relationship to TSH or total T4 levels. In Inuit adults, only highly chlorinated PCBs, including PCB180, were negatively associated with TSH, PCB118 and PCB153 were negatively associated with total T3 and no associations with Free-T4 were found.[18] The researchers found that when adjusted for

other families of contaminants, PCB118 negatively associated with Free-T4 and positively associated with TSH.[18] Zani et al[33] reported a weak inverse correlation between serum levels of total PCBs and Free-T3, Free-T4 and TSH in an Italian population but no associations were found when corrected for covariates.

The results reported in this study, however, are somewhat consistent with recent studies published on participants selected from the PBB Michigan register.[17,19] Jacobson et al[19] found that PCB congeners 118, 138, 153, and 180 were associated with greater total and Free-T4, and total T3 among women in lipid-standardized models, with greater estimates for PCB118 for total T4 and T3. Curtis et al[17] observed that PCBs were significantly associated with higher Free-T4 levels and found no associations for Free-T3 or TSH. The lack of findings with the other frequently detected PCBs in this study may be a result of the lower number of participants, n = 59 versus n = 551 and n = 715 and the lower mean concentration of PCB congeners, approximate one third that of the Michigan cohort. Alternatively, the association with PCB118 may be a consequence of the greater toxicity associated with dioxin like PCBs, with PCB118 being the only coplanar dioxin like PCB frequently detected in this study.

4.5 Other findings

Previous studies have reported that eGFR and C reactive protein were significantly higher in PCOS cases than controls and that there was positive correlation between elevated eGFR and C reactive protein.[43] In this study, PCOS cases had significantly lower eGFR compared to controls, there was no difference for CRP between cases and controls and no significant correlation between eGFR and CRP was observed (Table S2.). However, both eGFR and CRP negatively correlated with the frequently detected PCB congeners in this study. Although the main mode of elimination in PCBs is via feces, they and their metabolites have been found in urine.[4] Thus,

decreased eGFR in PCOS cases could potentially result in some increase in serum concentrations of PCBs, although PCB geometric means between cases and controls did not differ in this study.

4.6 Study strengths and limitations

The strengths of this study lie in the study design of a homogeneous age, ethnicity, and BMI matched PCOS cases and controls, the measurement of a range of metabolic, steroid, and thyroid hormone outcomes, and the representation of a potential sensitive subpopulation to exogenous exposure to persistent organic pollutants of environmental relevance reported to have endocrine disrupting effects.

The limitations of the study include the small sample size which may have resulted in insufficient statistical power to detect differences between cases and controls, missing data for some metabolic outcomes and hormone levels, and the chance finding of significance due to the large number of statistical analyses performed on the data set. Correlations and potential associations between PCB28, PCB52 and PCB101, and metabolic and hormonal outcomes were unable to be performed due to low detection frequency in PCOS and controls. To try to account for these PCB concentrations, the authors calculated a ∑PCB variable. Although analysis of potential covariates was considered, this was limited to age, smoking, BMI, eGFR, albumin and total serum lipids. Smoking was the only potential exposure source considered in this cohort. Regression models were not adjusted for other potential exposure sources as this data was not available. In addition, other covariates, such as additional environmental pollutants previously detected in this cohort,[26] were not considered. Whilst a study strength was that all participants were Caucasian and from a restricted geographical area, equally this is a limitation as the results may not be translatable to other ethnicities. Consequently, the results should be considered as

exploratory and to add to the current literature for hypothesis generation for future research within epidemiological studies.

5. Conclusion

Using an age, ethnicity, and BMI matched case-control study design, we found that the concentrations of PCBs did not differ between PCOS cases and controls. The few significant findings appear to be congener specific rather than linked to total PCB congener concentration. In addition, the association of PCB congener concentrations on a range of metabolic outcomes, and steroid and hormone levels examined in this cohort, do not suggest that PCOS patients are more adversely at risk to PCB exposure. This research highlights the need to further substantiate the effects of PCBs in larger cohorts on metabolic outcomes and hormone levels.

The relationship between PCBs and metabolic and hormone outcomes are complex. Specific PCB congeners have different physiochemical properties resulting in different direct and indirect effects including estrogenic/antiestrogenic and androgenic/antiandrogenic effects against specific hormone receptors. This is further complicated by the range of physiology of hormone levels between individuals and in metabolic states such as PCOS and potential lifelong exposure to vast mixtures of chemicals. Thus, further studies are required to elucidate the association of PCB concentrations with metabolic and hormone parameters and their impacts on the etiology and pathophysiology of PCOS.

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Ethics

This study was conducted in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). Ethical approval was obtained by The Yorkshire and The Humber NRES ethical committee, UK (approval number 02/03/043) and all participants gave their written informed consent.

Declaration of competing interest

All authors have no competing interests to declare, financial or otherwise.

Data access

The data that support the findings of this study are available from the corresponding author, [EB], upon reasonable request.

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Appendix - Supplementary material

Supplementary Table S1. Pearson correlation coefficients for PCB concentrations.

	lnPCB118 (ng/g lipid)	lnPCB138 (ng/g lipid)	lnPCB153 (ng/g lipid)	lnPCB180 (ng/g lipid)	ln∑PCB (ng/g lipid)
lnPCB118 (ng/g lipid)	1				
lnPCB138 (ng/g lipid)	.794**	1			
lnPCB153 (ng/g lipid)	.753**	.972**	1		
lnPCB180 (ng/g lipid)	.655**	.901**	.926**	1	
ln∑PCB (ng/g lipid)	.777**	.958**	.971**	.935**	1

ln, natural log transformation; PCB, polychlorinated biphenyl; **, $p < 0.01$; *, $p < 0.05$

Supplementary Table S2. Pearson correlation coefficients for PCBs and age, albumin, BMI, total serum lipids, eGFR and CRP.

	lnPCB118 (ng/g lipid)	lnPCB138 (ng/g lipid)	lnPCB153 (ng/g lipid)	lnPCB180 (ng/g lipid)	ln∑PCB (ng/g lipid)	Age (Years)	Albumin (g/L)	BM1 (kg/m^2)	Total Serum Lipids	lneGFR (mL/min/ $1.73m^2$)	lnCRP (mg/L)
Age (Years)	.404**	.594**	.594**	.656**	.636**	1					
Albumin (g/L)	0.198	0.124	0.109	0.123	0.083	-0.140	1				
BMI (kg/m^2)	-0.047	-0.056	-0.061	-0.118	-0.071	0.182	-0.124	1			
Total Serum Lipids (mg/dL)	-0.091	0.097	0.137	0.041	0.113	0.145	-0.194	.330*	1		
lneGFR (mL/min/ $1.73m^2$)	-.357**	-.306*	-.343**	-.335*	-.335*	-0.235	-0.196	-0.116	-0.211	1	
lnCRP (mg/L)	-.309*	-.277*	-.267*	-.296*	-0.222	-0.046	-0.199	.382**	.330*	0.022	1

ln, natural log transformation; PCB, polychlorinated biphenyl; BMI, body mass index; eGFR, estimated glomerular filtration rate; CRP, C reactive protein; **, $p < 0.01$; *, $p < 0.05$

Supplementary Table S3. Pearson correlation coefficients for PCBs and sex steroid concentrations.

	InPCB 118 (ng/g lipid)	InPCB 138 (ng/g lipid)	InPCB 153 (ng/g lipid)	InPCB 180 (ng/g lipid)	∑PCB (ng/g lipid)	InTestosterone (nmol/L)	InEstradiol (pmol/L)	Androstenedione (nmol/L)	InSHBG (nmol/L)	InFAI
InTestosterone (nmol/L)	0.068	-0.088	-0.061	-0.037	-0.085	1				
InEstradiol (pmol/L)	-0.064	-0.034	0.002	0.084	-0.003	.337*	1			
Androstenedione (nmol/L)	0.068	-0.168	-0.180	-0.213	-0.216	.795**	0.154	1		
InSHBG (nmol/L)	0.119	0.212	.270*	.362**	.294*	-0.121	0.306	-.417**	1	
InFAI	-0.079	-0.212	-0.239	-.298*	-.273*	.603**	-0.068	.733**	-.865**	1

In, natural log transformation; PCB, polychlorinated biphenyl; SHBG, sex hormone binding globulin; FAI, free androgen index; **, $p < 0.01$; *, $p < 0.05$

Supplementary Table S4. Pearson correlation coefficients for PCBs and metabolic outcomes.

	InPCB118 (ng/g lipid)	InPCB138 (ng/g lipid)	InPCB153 (ng/g lipid)	InPCB180 (ng/g lipid)	InΣPCB (ng/g lipid)	Fasting blood glucose (nmol/L)	InInsulin (µIU/ml)	InHOMA-IR	HbA1c (mmol/mol)
Fasting blood glucose (nmol/L)	0.063	0.142	0.136	0.140	0.127	1			
InInsulin (µIU/ml)	-0.180	-0.114	-0.126	-0.192	-0.121	0.251	1		
InHOMA-IR	-0.126	-0.105	-0.113	-0.188	-0.127	0.365**	0.819**	1	
HbA1c (mmol/mol)	-0.022	-0.083	-0.052	-0.073	-0.067	0.219	0.368**	0.380**	1

In, natural log transformation; PCB, polychlorinated biphenyl; HOMA-IR, homeostatic model assessment for insulin resistance; HbA1c, glycosylated hemoglobin A1c; **, $p < 0.01$

Supplementary Table S5. Pearson and Spearman correlation coefficients for PCBs and thyroid hormone levels.

	InPCB118 (ng/g lipid)	InPCB138 (ng/g lipid)	InPCB153 (ng/g lipid)	InPCB180 (ng/g lipid)	InΣPCB (ng/g lipid)	TSH (mU/L)	InFree-T3 (pmol/L)	Free-T4 (pmol/L)
TSH (mU/L)[a]	0.128	0.013	-0.033	-0.059	-0.029	1		
InFree-T3 (pmol/L)[a]	-0.206	-0.075	-0.065	-0.074	-0.087	-0.021	1	
Free-T4 (pmol/L)[b]	.311*	0.158	0.142	0.073	0.158	0.147	0.278	1

In, natural log transformation; PCB, polychlorinated biphenyl; TSH, thyroid stimulating hormone; Free-T4, free-thyroxine; Free-T3, free-triiodothyronine; *, $p < 0.05$; [a], Pearson correlation, [b], Spearman

Tables 1 - 6

Table 1. Summary of PCB serum concentrations (ng/g lipid) in study cohort.

PCB Congener	Description	n (%)	GM	Range	LOR
PCB28 2,4,4'-Trichlorobiphenyl	CP1 PP	15 (25)	9.67	<1.0-9.67	1
PCB52 2,2',5,5'- Tetrachlorobiphenyl	4CL 2M	4 (6.8)	1.97	<1.0-23.24	1
PCB101 2,2',4,5,5'- Pentachlorobiphenyl	4CL 2M	26 (44)	2.63	<0.8-3.76	1
PCB118 2,3',4,4',5- Pentachlorobiphenyl	CP1 4CL PP 2M	59 (100)	6.2	2.6-13.5	0.1
PCB138 2,2',3,4,4',5'- Hexachlorobipheny	4CL PP 2M	59 (100)	10.03	3.33-31.38	0.15
PCB153 2,2',4,4',5,5'- Hexachlorobiphenyl	4CL PP 2M	59 (100)	13.12	3.0-36.97	0.3
PCB180 2,2',3,4,4',5,5'- Heptachlorobiphenyl	4CL PP 2M	59 (100)	9.17	3.33-29.18	0.2

PCB, polychlorinated biphenyl; CP0, coplanar non-ortho; CP1, coplanar mono-ortho; PP, both para positions (4, 4') chlorinated, 4CL, ≥ 4 chlorine atoms; 2M, ≥ 2 meta positions (3, 3', 5, and 5') chlorinated; n, sample size; GM, geometric mean; LOR, limit of reporting

Table 2. PCB concentrations for PCOS patients and controls.

	Control (n=30)		PCOS (n=29)		
	Mean	n	Mean	n	p value
PCB118 GM (95% CI)	5.0 (4.5 - 5.7)	30	5.4 (4.7 - 6.2)	29	0.470 [a]
PCB138 GM (95% CI)	11.2 (9.4 - 13.3)	30	10.1 (8.3 - 12.2)	29	0.423 [a]
PCB153 GM (95% CI)	14.3 (11.5 - 17.6)	30	12.2 (9.7 - 15.4)	29	0.324 [a]
PCB180 GM (95% CI)	12.9 (10.7 - 15.6)	30	10.7 (8.7 - 13.1)	29	0.170 [a]
ΣPCB GM (95% CI)	46.1 (39.0 - 54.5)	30	41.7 (34.6 - 50.2)	29	0.407 [a]

GM, geometric mean; Cl, confidence interval; PCOS, polycystic ovarian syndrome; n, sample size;

[a], T-test

Table 3. Demographics, hormone, and biochemistry endpoints for PCOS patients and controls.

	Control (n=30)			PCOS (n=29)			
	Mean	± SD	n	Mean	± SD	n	p value
Age (Years)	32.6	4.7	30	30.9	4.8	29	0.179 [a]
BMI (kg/m^2)	25.5	3.6	30	26.0	3.8	29	0.563 [a]
Menarche (Years)	13.1	1.9	30	12.9	1.2	29	0.685 [a]
Smoker (%)	20.0			0			0.024 [c*]
Fasting blood glucose (nmol/L)	4.8	0.3	30	4.6	0.4	28	0.058 [a]
HOMA-IR	1.7	1.0	30	2.0	1.6	29	0.588 [a]
Insulin (µIU/mL)	7.7	4.0	30	8.1	4.7	29	0.786 [a]
HbA1c (mmol/mol)	31.8	2.6	27	32.0	3.3	27	0.749 [a]
Androstenedione (nmol/L)	2.5	1.2	22	4.1	1.5	25	0.0004 [a*]
Testosterone (nmol/L)	0.8	0.4	28	1.1	0.5	26	0.006 [a*]
SHBG (nmol/L)	109.7	83.7	29	64.6	51.4	27	0.042 [a*]
FAI	1.7	3.3	28	3.1	2.9	26	0.004 [a*]
Estradiol (pmol/L)	449.6	294.0	18	431.0	463.1	19	0.366 [a]
TSH (mU/L)	2.2	1.0	29	2.1	0.7	26	0.655 [a]
Free-T3 (pmol/L)	4.8	0.7	27	4.8	0.7	23	0.949 [a]
Free-T4 (pmol/L)	11.1	1.3	27	11.5	2.2	23	0.511 [b]
eGFR (mL/min/1.73 m^2)	97.4	18.2	29	88.1	10.3	27	0.037 [a*]
CRP (mg/L)	2.5	2.3	28	2.7	2.6	27	0.861 [a]
Albumin (g/L)	40.0	2.5	29	40.7	3.5	27	0.362 [a]
TC (mmol/L)	4.8	0.8	29	4.7	1.1	27	0.556 [a]
TG (mmol/L)	1.0	0.5	29	1.3	0.7	27	0.117 [b]
HDL-c (mmol/L)	1.7	0.3	26	1.4	0.4	21	0.010 [b*]
LDL-c (mmol/L)	2.7	0.6	26	2.6	0.9	21	0.566 [a]
Total serum lipids (mg/dL)	570.4	104.5	29	582.5	134.2	27	0.707 [a]

BMI, body mass index; HOMA-IR, homeostatic model assessment for insulin resistance; HbA1c, glycosylated hemoglobin A1c; SHBG, sex hormone binding globulin; FAI, free androgen index; TSH, thyroid stimulating hormone; Free-T3, free-triiodothyronine; Free-T4, free-thyroxine; eGFR, estimated glomerular filtration rate; CRP, C reactive protein; TC, total cholesterol; TG, triglycerides; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; PCOS, polycystic ovarian syndrome; SD, standard deviation; n, sample size; [a], T-test; [b], Mann-Whitney U test; [c], Pearson Chi-square, *, statistically significant result $p < 0.05$

Table 4. Associations between metabolic outcomes and serum PCB concentrations, adjusted for BMI and age.

Exposure variable	**Outcome variable, β (95% CI)**							
	Fasting blood glucose, nmol/L[a]		ln(HOMA-IR)[a]		ln(Insulin, µIU/ml)[a]		HbA1c, mmol/mol[a]	
	PCOS	Controls	PCOS	Controls	PCOS	Controls	PCOS	Controls
PCB118 (ng/mg Lipid)	0.002 (-0.075 ,0.079)	-0.043 (-0.128 ,0.041)	0.006 (-0.069, 0.081)	-0.079 (-2.356, 1.157)	-0.006 (-0.079, 0.067)	-0.074 (-0.181, 0.032)	-0.038 (-0.565, 0.489)	-0.360 (-1.00, 0.284)
PCB138 (ng/mg Lipid)	-0.007 (-0.034 ,0.02)	-0.005 (-0.044, 0.034)	0.001 (-0.025, 0.028)	-0.023 (-0.077, 0.032)	0.006 (-0.02, 0.032)	-0.022 (-0.071, 0.027)	-0.073 (-0.259, 0.113)	-0.149 (-0.446, 0.147)
PCB153 (ng/mg Lipid)	-0.006 (-0.025, 0.012)	-0.004 (-0.03, 0.022)	0.003 (-0.015, 0.021)	-0.022 (-0.058, 0.014)	0.006 (-0.011, 0.023)	-0.022 (-0.054, 0.011)	-0.059 (-0.183, 0.065)	-0.013 (-0.213, 0.187)
PCB180 (ng/mg Lipid)	-0.015 (-0.042, 0.013)	-0.002 (-0.039, 0.035)	-0.001 (-0.029, 0.026)	-0.027 (-0.079, 0.024)	0.004 (-0.023, 0.03)	-0.028 (-0.074, 0.018)	-0.142 (-0.325, 0.041)	0.002 (-0.271, 0.275)
ΣPCB (ng/mg Lipid)	-0.003 (-0.011, 0.004)	-0.002 (-0.012, 0.009)	0.0001 (-0.007, 0.008)	-0.007 (-0.022, 0.007)	0.002 (-0.005, 0.01)	-0.007 (-0.02, 0.006)	-0.035 (-0.086, 0.016)	-0.015 (-0.095, 0.065)

β, regression coefficients; Cl, confidence interval; In, natural log transformation; HOMA-IR, homeostatic model assessment for insulin resistance; HbA1c, glycosylated hemoglobin A1c; [a], multivariable linear regression; PCOS, polycystic ovarian syndrome; PCB, polychlorinated biphenyl

Table 5. Associations between steroid hormones and serum PCB concentrations, adjusted for BMI, age and serum albumin.

Exposure variable	**Outcome variable, β (95% CI)**									
	Androstenedione, nmol/L[a]		ln(Testosterone, nmol/L)[a]		ln(SHBG, nmol/L)[a]		FAI[b]		Estradiol, pmol/L[c]	
	PCOS	Controls	PCOS	Controls	PCOS	Controls	PCOS	Controls	PCOS	Controls
PCB118 (ng/mg Lipid)	0.102 (-0.281, 0.485)	-0.081 (-0.448, 0.286)	-0.032 (-0.129, 0.066)	0.017 (-0.118, 0.152)	-0.099 (-0.231, 0.032)	0.197 (0.000, 0.395)	0.14 (-0.53, 0.83)	-0.068 (-0.26, 0.12)	-46 (-151, 58)	-92 (-199, 15)
PCB138 (ng/mg Lipid)	0.031 (-0.126, 0.187)	-0.016 (-0.164, 0.132)	-0.023 (-0.057, 0.012)	0.014 (-0.046, 0.073)	-0.033 (-0.081, 0.016)	0.045 (-0.049, 0.139)	0.03 (-0.19, 0.27)	-0.009 (-0.09, 0.077)	-28.8 (-61.7, 4.1)	-37.9 (-76.8, 0.96)
PCB153 (ng/mg Lipid)	0.043 (-0.066, 0.153)	-0.029 (-0.13, 0.071)	-0.013 (-0.037, 0.01)	0.017 (-0.022, 0.056)	-0.026 (-0.058, 0.006)	0.053 (-0.007, 0.113)	0.03 (-0.13, 0.19)	-0.021 (-0.07, 0.03)	-19.5 (-41.6, 2.56)	-19.5 (-46.4, 7.2)
PCB180 (ng/mg Lipid)	0.065 (-0.095, 0.225)	-0.06 (-0.202, 0.082)	-0.006 (-0.045, 0.033)	0.033 (-0.02, 0.087)	-0.025 (-0.079, 0.029)	0.063 (-0.023, 0.148)	0.05 (-0.24, 0.34)	-0.02 (-0.09, 0.04)	-30.1 (-67.2, 6.8)	-13.2 (-49.2, 22.6)
ΣPCB (ng/mg Lipid)	0.001 (-0.043, 0.044)	-0.009 (-0.05, 0.032)	-0.007 (-0.017, 0.002)	0.007 (-0.009, 0.022)	-0.009 (-0.022, 0.005)	0.021 (-0.003, 0.045)	0.01 (-0.05, 0.08)	-0.008 (-0.029, 0.012)	-9.17 (-18.39, 0.055)	-7.81 (-18.55, 2.93)

β, regression coefficients; Cl, confidence interval; In, natural log transformation; SHBG, sex hormone binding globulin; FAI, free androgen index; [a], multivariable linear regression; [b], Non-parametric quantile regression; [c], Tobit regression; PCOS, polycystic ovarian syndrome; PCB, polychlorinated biphenyl

Table 6. Associations between thyroid hormone concentrations and serum PCB concentrations, adjusted for serum albumin and smoking.

Exposure variable	**Outcome variable, β (95% CI)**					
	TSH, mU/L[a]		ln(Free-T4, pmol/L)[a]		ln(Free-T3, pmol/L)[a]	
	PCOS	Controls	PCOS	Controls	PCOS	Controls
PCB118 (ng/mg Lipid)	0.008 (-0.116, 0.133)	0.067 (-0.169, 0.302)	0.022 (-0.006, 0.050)	0.016 (-0.013, 0.045)	-0.009 (-0.032, 0.015)	-0.011 (-0.046, 0.024)
PCB138 (ng/mg Lipid)	-0.016 (-0.061, 0.028)	0.017 (-0.068, 0.101)	0.007 (-0.003, 0.016)	0.004 (-0.006, 0.015)	-0.001 (-0.009, 0.008)	-0.001 (-0.014, 0.011)
PCB153 (ng/mg Lipid)	-0.013 (-0.044, 0.017)	0.004 (-0.053, 0.060)	0.004 (-0.003, 0.011)	0.001 (-0.006, 0.009)	0.000 (-0.006, 0.005)	-0.001 (-0.009, 0.008)
PCB180 (ng/mg Lipid)	-0.022 (-0.065, 0.021)	-0.015 (-0.085, 0.056)	0.002 (-0.008, 0.012)	0.002 (-0.007, 0.011)	-0.002 (-0.01, 0.006)	0.002 (-0.008, 0.013)
ΣPCB (ng/mg Lipid)	-0.006 (-0.018, 0.006)	0.001 (-0.02, 0.023)	0.002 (-0.001, 0.004)	0.001 (-0.002, 0.004)	0.000 (-0.003, 0.002)	-0.000 (-0.003, 0.003)

β, regression coefficients; Cl, confidence interval; In, natural log transformation; TSH, thyroid stimulating hormone; Free-T4, free-thyroxine; Free-T3, free-triiodothyronine; [a], multivariable linear regression; PCOS, polycystic ovarian syndrome; PCB, polychlorinated biphenyl