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Exploring the potential association between brominated diphenyl ethers, polychlorinated biphenyls, organochlorine pesticides, perfluorinated compounds, phthalates, and bisphenol a in polycystic ovary syndrome: a case–control study

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Abstract

Background: Polycystic Ovary Syndrome (PCOS) is an endocrine-metabolic disorder that affects approximately 6-10% of women of child-bearing age. Although preliminary studies suggest that certain pollutants may act as endocrine disruptors in animals, little is known about their potential association with PCOS. The objective of this case-control pilot study is to determine whether women with PCOS have higher concentrations of specific environmental contaminants compared to women who have not developed PCOS.

Methods: Fifty-two PCOS case-patients (diagnosed using the National Institutes of Health 1990 definition) and 50 controls were recruited in 2007–2008, from an urban academic medical center in Los Angeles, CA. Brominated diphenyl ethers, polychlorinated biphenyls (PCBs), organochlorine pesticides, and perfluorinated compounds (PFCs) were measured in serum, and phthalates metabolites and bisphenol A (BPA) in urine.

Results: PCOS case-patients had significantly higher geometric mean (GM) serum concentrations of two PFCs: perfluorooctanoate (PFOA) (GM_{cases} = 4.1 μg/L, $GM_{controls}$ = 2.3 μg/L; p = 0.001) and perfluorooctane sulfonate (PFOS) (GM_{cases} = 8.2 μg/L, $GM_{controls}$ = 4.9 μg/L; p = 0.01), and lower urinary concentrations of monobenzyl phthalate (mBzP) (GM_{cases} = 7.5 μg/g creatinine, $GM_{controls}$ = 11.7 μg/g creatinine; p = 0.02). Logistic regression, controlling for body mass index, age and race, identified an increased likelihood of PCOS in subjects with higher serum concentrations of PFOA and PFOS (adjusted-ORs = 5.8–6.9, $p < 0.05$), and with lower urine concentrations of mBzP and mono-n-butyl phthalate (mBP) (aORs = 0.14–0.25, $p < 0.05$).

Conclusions: Our data suggest that PCOS case-patients may differ from controls in their environmental contaminant profile. PCOS subjects had higher serum concentrations of two PFCs, PFOA and PFOS, and lower urine concentrations of mBP and mBzP. Future studies are needed to confirm these preliminary findings and determine if these chemicals or their precursors may have a role in the pathogenesis of PCOS.

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Background

Polycystic ovary syndrome (PCOS) is an endocrine-metabolic disorder that affects approximately 6-10% of women of child bearing age [1-4], approximately 4 million women in the United States, and is the most frequent cause of oligo-anovulatory infertility [5]. PCOS is characterized by ovulatory dysfunction, hirsutism, or hyperandrogenemia and is associated with insulin resistance, hyperinsulinemia, type 2 diabetes mellitus, and endometrial carcinoma [4,6]. The annual cost of evaluating and providing care for PCOS to reproductive-aged women in the United States alone exceeds $4.3 billion [7].

Some pollutants may disrupt endocrine processes, but little is known about the effects of environmental contaminants on the development of PCOS. Organochlorine pesticides (OCPs) and bisphenol A (BPA) have been postulated as potential xenohormones in women by mimicking estrogen action and/or antagonizing testosterone action and potentially altering the secretions of follicle-stimulating hormone and luteinizing hormone [8]. I*n vitro* and animal studies have implicated polybrominated diphenyl ethers (PBDEs), phthalates, polychlorinated biphenyls (PCBs) and BPA as endocrine disruptors [9-14]. All of these industrial chemicals can be detected in food, water, or air, and diet is an important route of human exposure [15].

Considering the high prevalence of PCOS, the existence of an association between exposure to environmental chemicals and disease could have significant public health and economic impact. We investigated whether an increase in the likelihood of being diagnosed with PCOS may be associated with exposure to 36 PCBs, 9 OCPs, 11 PBDEs, 8 PFCs, 8 phthalates, and BPA as measured by concentrations in serum or urine.

Methods

Participants and setting

Between March 2007 and May 2008 the Center for Androgen-related Research and Discovery at Cedars-Sinai Medical Center (CSMC) in Los Angeles, CA recruited fifty-two women with PCOS and fifty controls through media advertisements and subspecialty clinics. Most (>90%) of PCOS patients agreed to store blood and urine samples for future studies. As part of the CSMC study, controls volunteered in response to advertising in the surrounding community and university for "healthy" women aged 25–45 years. All women were aged 18– 45 years. Both PCOS patients and controls provided CSMC with information about their age, race, ethnicity, virilization and body mass index which are associated with case status. Participants enrolled in the CSMC study provided written informed consent to allow CSMC to provide Centers for Disease Control and Prevention (CDC) with anonymized data and samples for our nested pilot study. This study was approved by the Internal Review Board of the Centers for Disease Control and Prevention (CDC protocol #4918).

Exclusion criteria included concurrent pregnancy, the use of hormonal (including oral contraceptives) or other medications for the prior three months, diabetes, menopause, and inability to provide written consent.

We defined case-patients based on the strict National Institutes of Health 1990 criteria for PCOS, specifically including anovulation or oligo-ovulation; clinical (i.e., a modified Ferriman-Gallwey [mFG] hirsutism score >6) or laboratory evidence of hyperandrogenism; and the exclusion of related disorders (i.e. thyroid dysfunction, hyperprolactinemia, non-classic adrenal hyperplasia, androgen-secreting tumors, etc.), as previously described [16]. We defined controls as healthy women with long-term regular and predictable menstrual cycles, without hirsutism (i.e. mFG scores of ≤2), and without clinical and laboratory evidence of hyperandrogenism or other hormonal dysfunction.

Measures, sample collection and handling

Recruited participants provided single spot urine and blood samples at CSMC. To avoid environmental contamination, participants were asked to open the urine containers when ready to provide a sample and to avoid touching the inside of the container. Serum samples where obtained by venipuncture performed using standard protocol and precautions, and the samples drawn into one 10 ml red top Vacutainer® tube per participant. Tubes were placed upright and blood allowed to clot at room temperature for 60 min, after which they were centrifuged at 3,000 rpm (~1000 g) for 10 min. Using a disposable pipet, the serum was transferred to CDC-provided containers (10 mL amber glass bottle with Teflon-lined screw cap [Wheaton, Millville, NJ] Serum vial for serum PBDE content and 2 mL Nalgene® [Rochester, NY] cryovials for serum PFCs and lipids content). The serum PBDE vials had been previously cleaned in a laboratory dish washer and baked at 300°C overnight and the screw caps had been rinsed with methanol [17]. After recapping, each container was frozen upright in a CDC-provided cardboard storage boxes at −70°C until shipping.

First morning void urine samples were collected in individually wrapped collection cups provided by the CDC. At the time of collection participants were asked to wash their hands with soap and water, to collect at least 10–15 ml of urine in the cup, to not remove the cap from the cup until ready to void, and to not touch the inside of the cup or cap. Using a sterile disposable plastic pipet, urine samples were transferred into prelabeled 5 ml Nalgene® cryovials (for urinary Phthalates/

BPA content) and 2 ml Nalgene® cryovials (for urinary creatinine content). The urinary samples were then placed in CDC-provided storage boxes and frozen at −20°C until shipping.

All sample collection vials were labeled with pre-printed bar-coded labels provided by the CDC, and the date and time of collection was added using a permanent marker. Operators used disposable powder-free nitrile gloves and surgical masks during sample collection and processing, and all collection and processing supplies were provided by the CDC to maximize the integrity of the samples. All sample processing was performed in a fume hood lined with aluminum foil to minimize contamination by ambient dust, as PBDEs are well known contaminants in indoor dust. The laboratory space was further thoroughly cleaned before any laboratory work was undertaken to minimize any dust or particulate matter that could contaminate the samples. All samples were shipped to the CDC on dry ice and upon arrival stored at −70°C until the time of analysis [17].

Toxicologic analyses

Blood samples were analyzed for concentration of 36 PCBs, 9 OCPs, 11 PBDEs and 8 PFCs. Laboratory results of blood-agent concentration included both whole serum and lipid-adjusted concentration values of PCBs, OCPs and BDEs and serum only for PFCs.

Analytical determination of PCBs, OCPs and PBDEs in serum were performed by gas chromatography isotope dilution high resolution mass spectrometry, after solid phase extraction (SPE) and co-extracted lipid removal techniques [17]. Total cholesterol and triglycerides were measured using a Roche Hitachi Mod P Chemistry Analyzer (Roche Hitachi, Basel, Switzerland), using single-point, forced-through-zero calibration curves and Roche colorimetric methods as described in the product applications #11491458216 V15 (total cholesterol) and #04843673003 V13 (total triglycerides).

The measurements of PCBs, OCPs, and PBDEs were made in batches of twenty-four unknowns, three quality controls and three method blanks. The method blanks were used to track any analytical background during the sample preparation and the final analytical results were blank subtracted. The limits of detection (LODs) were calculated as standard deviations (SDs) of the method blanks analyzed in parallel with the unknowns after subtracting the average blank concentration or as the instrumental LOD in the absence of a detectable blank level (Additional file 1). The concentration ratio between 2,2′,4,4′,5-pentabromodipehnyl ether (BDE-99) and 2,2′,4,4′-tetrabromodipehnyl ether (BDE-47) can be used as an indicator of contamination during sample collection. The median ratio (BDE-99/BDE-47) in this study was 0.19 (range 0.10 – 0.36) which is consistent with a metabolized pattern and hence did not indicate contamination of indoor dust which is expected to have a ratio close to 1 (i.e. the concentration ratio between BDE-99 and BDE-47 in the commercial pentaBDE product used as a flame retardant).

We measured PFCs in serum using a modification of the on-line SPE coupled to high-performance liquid chromatography (HPLC)-isotope dilution tandem mass spectrometry (MS/MS) approach previously described in detail [18].

Urine samples were analyzed for 11 phthalate metabolites and for BPA (total concentrations) using a modification of the on-line SPE-high performance liquid chromatography-isotope dilution tandem mass spectrometry approaches described before [19,20]. Urinary creatinine, used to adjust for the dilution of the urine, was measured using an enzymatic reaction on a Roche Hitachi 912 chemistry analyzer (Roche Hitachi, Basel, Switzerland).

Statistical analyses

Epidemiologic analyses

Concentrations that were below the LOD were assigned a value equal to LOD/√2 [21]. No statistical analyses were conducted for compounds for which the majority (>50%) of samples had concentrations < LOD. We searched for PBDE, PCB, OCP, phthalate metabolites, PFCs and BPA concentrations that were significantly ($p < 0.05$) higher among PCOS case-patients compared to controls using Wilcoxon Rank-Sum.

Multivariate logistic regression analyses

We used the serum concentrations or urine concentrations of the chemicals to categorize exposures to the chemicals or their parent compounds. We split observations into tertiles based on chemical concentrations then performed multivariable logistic regression analyses to explore associations between the concentrations of the chemicals (in μg/L for PFCs; lipid-adjusted for PCBs, OCPs and PBDEs and creatinine-adjusted for phthalate metabolites and BPA) and PCOS case status after adjusting for potential confounders (i.e. age [divided into 5 categories], body mass index [BMI], and White race [compared to all other races]). This multivariable logistic regression model is represented as PCOS $(0/1) = \alpha + \beta_1$ (chemical concentration tertile) + β_2(age) + β_3(BMI) + β_4 (race). Hispanic ethnicity was not significantly related to PCOS when tested in preliminary analyses by logistic regression alone or when controlling for race, BMI, or age; therefore we did not statistically control for Hispanic ethnicity in the final models to conserve power. Due to the high ratio of variables in the model to study participants we calculated exact adjusted odds ratios, exact p values, and exact confidence intervals using the network method [22]. All statistical analyses were conducted

using SAS v9.3 (Cary, NC). More than 50% of the samples had serum or urine concentrations < LOD for 22 PCBs, 3OCPs, 2 phthalate metabolites, and 2 PFCs; thus, no further analyses were conducted for these compounds (Additional file 2).

Results

Comparing the 52 PCOS case-patients and 50 controls, we observed that women with PCOS had a greater mean BMI (OR = 1.1; 95% CI = 1.0–1.2) and were younger (OR = 0.7; 95% CI = 0.5–0.9) than controls (Table 1). Forty-four (85%) of 52 PCOS case-patients and 27 (54%) controls were white, 6 (12%) case-patients and 16 (32%) controls were Black, and two (4%) case-patients and six (12%) controls were Asian (p = 0.003). Thirty (58%) of 52 PCOS case-patients were Hispanic compared to 37 (74%) controls (p = 0.06). Case-patients and controls had similar total cholesterol (median 188 mg/dL [IQR 160–213]) and triglycerides (median 88 mg/dL [IQR 62–137]) (Table 2).

Overall, more than 50% of participants had detectable serum or urine concentrations of 6 PBDEs, 14 PCBs, 6 OCPs, 11 phthalate metabolites, 4 PFCs, and BPA. Correlations between concentration values can be found in (Additional file 3).

Perfluorinated compounds

PCOS case-patients had perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) geometric mean serum-concentration higher than controls (4.1 μg/L vs. 2.3 μg/L, p = 0.001; and 8.2 μg/L vs. 4.9 μg/L, p = 0.01, respectively) (Table 3). Participants were 6.9-fold more likely to have PCOS if they had PFOA concentrations in the highest tertile (geometric mean = 5.5 μg/L, range = 4.1–13.4 μg/L) when compared to those in the lowest tertile (geometric mean = 1.6 μg/L, range = 0.2–2.6 μg/L). Participants were also 5.8-fold more likely to have PCOS if they had PFOS concentrations in the highest tertile (geometric mean = 12.5 μg/L, range = 8.6–27.9 μg/L) (Table 4).

Polychlorinated biphenyls

Participants were 5.79-7.5 times more likely to have PCOS if they had PCB 153, 170, 180, 183, or 196 and 203 whole weight (pg/mL serum) concentrations in the middle tertile (but not the highest tertile), when compared to those in the lowest tertile (Table 4). A similar pattern also occurred among lipid-adjusted measurements of PCB 170, 180, and 196 and 203 concentrations. PCOS case-patients had PCB180 geometric mean lipid-adjusted concentrations (4.3 ng/g) *lower* than controls (5.3 ng/g) (p = 0.04) (Table 3).

Table 1 Minimum, maximum, mean and standard deviation values for body mass index (BMI, in Kg/m^2) and age (in years) at time of recruitment for cases and controls

		Mean (Range)	S.D.	t (control–case)	p-value[a]
BMI	Cases	32.82 (18.9-51.2)	8.32	**−3.427**	0.001
	Controls	27.62 (17.5-47.1)	6.91		
AGE	Cases	28.12 (18.0-45.0)	6.04	**2.673**	0.009
	Controls	31.84 (20.0-45.0)	7.94		

[a]p-value comparing mean of cases to mean of controls.

Table 2 Minimum, maximum, mean, median, and interquartile range values for serum whole weight, total lipid, cholesterol and triglycerides for cases and controls

		Mean	Range	Median	Interquartile range
Serum weight (g)	Cases	1.97	1.86-2.04	1.99	1.94-2.00
	Controls	1.97	1.87-2.04	1.99	1.96-2.01
Total lipid (mg/dL)	Cases	607.09	404.9-1002.8	590.3	519.48-684.98
	Controls	589.82	347.8-830.2	560.4	491.05-691.53
Cholesterol (mg/dL)	Cases	1.89	129-287	186.5	165-213
	Controls	1.88	98-281	187.5	160-214
Triglycerides (mg/dL)	Cases	114.75	37-457	96.5	67.25-138.00
	Controls	99.8	24-223	77.0	58.25-135.25

Phthalates

PCOS case-patients had monobenzyl phthalate (mBzP) geometric mean creatinine-adjusted urine concentrations (7.5 μg/g creatinine) *lower* than controls (11.7 μg/g creatinine) (p = 0.02) (Table 5). After controlling for BMI, age, and race, participants were *less* likely to have PCOS if they had creatinine-adjusted urine concentrations of mBzP or mono-n-butyl phthalate (mBP) in the middle or highest tertile compared to the lowest and mono-2-ethylhexyl phthalate (mEHP), and monoethyl phthalate (mEP) in the middle (but not the highest) tertile compared to the lowest (Table 6).

Other findings

PCOS case-patients and controls had similar geometric mean lipid-adjusted PBDEs, and OCPs serum concentrations (Table 3), and urinary BPA concentrations (Table 5), with the exception of BB153 of which controls had higher concentrations than case-patients (Table 3). After controlling for BMI, age, and ethnicity, no increase in likelihood for PCOS was associated with any of the measured PBDEs, OCPs, or BPA, with one exception (Tables 4 & 6).

Discussion

Key results

Our findings suggest that increasing odds of PCOS case-status was associated with higher serum concentrations of two PFCs (PFOA and PFOS) and with several PCB congeners. PFOS, PFOA, PCBs and phthalates have all

Table 3 Serum and lipid-adjusted concentrations of brominated diphenyl ethers, polychlorinated biphenyls, and persistent organic pesticides, and perfluorinated compounds in polycystic ovary syndrome (PCOS) case-patients and controls

	Geometric mean whole weight concentration (pg/g serum)		Lipid-adjusted concentration (ng/g lipid)	
Compounds	**Case-patients**	**Controls**	**Case-patients**	**Controls**
Brominated (Bi-) Diphenyl Ethers				
2,4,4′-tribromodiphenyl ether (PBDE28)	10.8	9.9	1.9	1.8
2,2′,4,4′-tetrabromodiphenyl ether (PBDE47)	144.5	148.6	24.9	26.5
2,2′,4.4′,5-pentabromodiphenyl ether (PBDE99)	27.5	28.8	4.7	5.1
2,2′,4,4′,6-pentabromodiphenyl ether (PBDE100)	27.8	29.8	4.8	5.3
2,2′,4,4′,5,5′-hexabromobiphenyl (BB153)	3.3	6.0	0.6	1.0[a]
2,2′,4,4′,5,5′-hexabromodiphenyl (PBDE153)	29.7	34.3	5.1	6.1
Polychlorinated Biphenyls				
2,2′,4,4′,5-pentaCB (PCB99)	10.8	12.8	1.9	2.3
2,3,3′,4,4′-pentaCB (PCB105)	3.8	3.8	0.7	0.7
2,3′,4,4′,5-pentaCB (PCB118)	15.6	15.6	2.7	2.8
2,2′,3,4′,5,5′-hexaCB (PCB146)	4.2	5.0	0.7	0.9
2,2′,4,4′,5,5′-hexaCB (PCB153)	38.6	47.2	6.7	8.4
2,3,3′,4,4′,5-hexaCB (PCB156)	4.3	5.4	0.7	1.0
2,2′,3,4,4′,5′-hexaCB (PCB138-158)	31.5	35.7	5.4	6.4
2,2′,3,3′,4,4′,5-heptaCB (PCB170)	9.9	12.0	1.7	2.1
2,2′,3,4,4′,5,5′-heptaCB (PCB180)	25.1	29.7	4.3	5.3[b]
2,2′,3,4,4′,5′,6-heptaCB (PCB183)	3.8	4.4	0.7	0.8
2,2′,3,4′,5,5′,6-heptaCB (PCB187)	7.1	9.2	1.2	1.7
2,2′,3,3′,4,5,6,6′-octaCB (PCB199)	4.0	5.3	0.7	0.9
2,2′,3,3′,4,4′,5,5′-octaCB (PCB 194)	0.7	1.1	0.7	1.1
2,2′,3,3′,4,4′,5′,6-octaCB and 2,2′,3,4,4′,5,5′,6-octaCB (PCB196_203)	4.6	6.2	0.8	1.1
Persistent pesticides				
Hexachlorobenzene (HCB)	53.9	51.2	9.2	9.1
β-Hexachlorocyclohexane (B-HCCH)	16.0	16.1	4.2	4.4
Oxychlordane	18.6	23.1	3.2	4.1
Trans-Nonachlor	29.6	31.9	5.1	5.7
2,2-Bis(4-chlorophenyl)-1, 1-dichloroethene (PP DDE)	1217.5	1397.2	210.2	248.7
2,2-Bis(4-chlorophenyl-1, 1, 1-trichloroethane (PP DDT)	19.0	18.1	3.3	3.2
Perfluorinated Compounds	μg/L serum	μg/L serum		
Perfluorooctanoate (PFOA)	4.1	2.3[c]		
Perfluorooctane sulfonate (PFOS)	8.2	4.9[d]		
Perfluorohexane sulfonate (PFHxS)	1.1	0.7		
Perfluorononanoate (PFNA)	1.2	0.9		

[a]p = 0.02 where controls have higher BB153 levels than case-patients.
[b]p = 0.04 where controls have higher PCB180 levels than case-patients.
[c]p = 0.001 where case-patients have higher PFOA levels than controls.
[d]p = 0.01 where case-patients have higher PFOS levels than controls.

been implicated as endocrine disruptors and have been linked to other reproductive outcomes. Previous epidemiologic investigations suggest that exposure to PFCs may increase women's risk of early menopause [23], thyroid disease [24], and delayed pregnancy and subfecundity [25-27]. Exposure to certain PCBs has been associated with other reproductive health effects such as delayed pregnancy [28]. PCB exposure has also been linked to increased breast cancer [29], diabetes and thyroid disease [30] risk. This is the first

Table 4 Logistic regression for middle and highest tertile serum concentrations compared to the lowest tertile for brominated diphenyl ethers, polychlorinated biphenyls, organochlorine pesticides, and perfluorinated compounds

				Unadjusted concentrations							Lipid-adjusted concentrations						
Agent	**N Case**	**N Control**	**Tertile**	**OR**	**95% C.I.**	**p**	**Adj OR[a]**	**Exact 95% C.I**	**Exact p**	**N Case**	**N Control**	**OR**	**95% C.I.**	**p**	**Adj OR[a]**	**Exact 95% C.I**	**Exact p**
Brominated Diphenyl Ethers																	
2,4,4′-tribromodiphenyl ether (PBDE28)	15	19	Middle	0.79	0.30-2.05		0.54	0.14-1.90		19	16	1.32	0.51-3.41		1.04	0.29-3.68	
	20	14	Highest	1.43	0.55-3.72		0.95	0.27-3.31		15	18	1.00	0.38-2.68		0.69	0.17-2.64	
2,2′,4,4′-tetrabromodiphenyl ether (PBDE47)	17	17	Middle	1.00	0.39-2.59		0.67	0.18-2.30		16	18	0.79	0.30-2.05		0.65	0.17-2.38	
	18	16	Highest	1.12	0.43-2.91		0.70	0.21-2.76		18	16	1.00	0.39-2.59		0.73	0.20-2.60	
2,2′,4.4′,5-pentabromodiphenyl ether (PBDE99)	18	15	Middle	1.27	0.49-3.29		1.04	0.30-3.52		17	17	0.89	0.34-2.30		1.21	0.36-4.26	
	17	17	Highest	1.06	0.41-2.72		0.70	0.20-2.37		17	17	0.89	0.34-2.30		0.63	0.18-2.16	
2,2′,4,4′,6-pentabromodiphenyl ether (PBDE100)	18	16	Middle	1.00	0.39-2.59		0.68	0.18-2.38		20	14	1.43	0.55-3.72		0.90	0.25-3.14	
	16	18	Highest	0.79	0.31-2.05		0.65	0.17-2.40		15	19	0.79	0.30-2.05		0.66	0.17-2.50	
2,2′,4,4′,5,5′-hexabromobiphenyl ether (BB153)	23	13	Middle	1.24	0.47-3.25		1.26	0.35-4.60		17	12	0.85	0.32-2.26		1.10	0.30-4.14	
	9	23	Highest	**0.27**	0.10-0.77	0.01	0.31	0.04-2.15		10	23	**0.26**	0.10-0.69	0.01	0.49	0.08-2.90	
2,2′,4,4′,5,5′-hexabromodiphenyl ether (PBDE153)	19	16	Middle	1.06	0.41-2.72		1.29	0.39-4.45		17	17	0.70	0.27-1.83		1.03	0.30-3.65	
	15	18	Highest	0.74	0.28-1.94		1.11	0.31-4.04		15	19	0.55	0.21-1.44		1.01	0.27-3.94	
Polychlorinated Biphenyls																	
2,2′,4,4′,5-pentaCB (PCB99)	18	16	Middle	0.70	0.27-1.83		2.12	0.55-9.07		16	22	**0.31**	0.11-0.86	0.024	0.94	0.23-3.41	
	13	21	Highest	0.38	0.14-1.02	0.055	1.10	0.27-4.65		15	19	**0.34**	0.12-0.95	0.040	0.86	0.23-3.84	
2,3,3′,4,4′-pentaCB (PCB105)	19	15	Middle	1.00	0.38-2.60		1.19	0.34-4.26		16	16	0.86	0.34-2.19		0.92	0.27-3.14	
	14	20	Highest	0.55	0.21-1.45		0.79	0.22-2.83		15	16	0.80	0.31-2.07		1.16	0.34-4.09	
2,3′,4,4′,5-pentaCB (PCB118)	17	17	Middle	0.89	0.34-2.30		1.68	0.46-6.57		20	14	1.27	0.49-3.31		2.06	0.59-7.89	
	17	17	Highest	0.89	0.34-2.30		2.18	0.56-9.22		14	20	0.62	0.24-1.62		1.21	0.31-4.88	
2,2′,3,4′,5,5′-hexaCB (PCB146)	17	13	Middle	1.12	0.43-2.92		4.01	0.98-19.44	0.055	15	12	0.98	0.37-2.60		3.33	0.81-15.86	
	14	19	Highest	0.63	0.25-1.61		2.91	0.68-14.15		14	20	0.55	0.22-1.38		2.40	0.60-10.83	
2,2′,4,4′,5,5′-hexaCB (PCB153)	22	12	Middle	1.83	0.69-4.85		**5.79**	1.41-28.11	0.011	17	17	0.48	0.18-1.28		1.88	0.47-8.21	
	13	21	Highest	0.62	0.24-1.62		4.20	0.81-24.95		12	22	**0.26**	0.09-0.71	0.009	1.42	0.27-7.89	
2,3,3′,4,4′,5-hexaCB (PCB156)	21	14	Middle	1.33	0.51-3.46		3.40	0.91-14.21		20	14	1.05	0.40-2.78		2.25	0.62-8.79	
	13	20	Highest	0.58	0.22-1.52		4.50	0.76-31.05		13	22	0.44	0.16-1.15		2.58	0.45-16.22	
2,2′,3,4,4′,5′-hexaCB and 2,3,3′,4,4′,6-hexaCB (PCB138-158)	20	14	Middle	1.13	0.43-2.95		2.73	0.72-11.33		18	18	0.60	0.23-1.58		1.57	0.43-6.11	
	13	21	Highest	0.49	0.18-1.29		2.48	0.50-13.49		14	20	0.42	0.16-1.13		1.70	0.39-7.90	

Table 4 Logistic regression for middle and highest tertile serum concentrations compared to the lowest tertile for brominated diphenyl ethers, polychlorinated biphenyls, organochlorine pesticides, and perfluorinated compounds *(Continued)*

t4.39	2,2′,3,3′,4,4′,5-heptaCB (PCB170)	24	10	Middle	2.70	0.99-7.33	0.051	**6.88**	1.71-32.96	0.003	20	13	1.30	0.49-3.40	**5.13**	1.24-25.16	0.020
t4.40		12	22	Highest	0.61	0.23-1.62		4.04	0.64-29.31		13	21	0.52	0.20-1.36	4.97	0.78-37.25	
t4.41	2,2′,3,4,4′,5,5′-heptaCB (PCB180)	11	23	Middle	2.35	0.88-6.30		**6.42**	1.60-30.52	0.005	19	14	1.02	0.39-2.66	**4.48**	1.08-21.73	0.037
t4.42		21	13	Highest	0.70	0.27-1.83		5.21	0.88-36.37		13	21	0.46	0.18-1.22	5.93	0.88-48.77	0.073
t4.43	2,2′,3,4,4′,5′,6-heptaCB (PCB183)	22	12	Middle	1.83	0.69-4.85		**4.21**	1.11-18.26	0.017	21	12	1.65	0.63-4.36	3.49	0.94-14.53	0.065
t4.44		13	21	Highest	0.62	0.24-1.62		3.55	0.67-21.68		13	21	0.59	0.22-1.52	2.94	0.59-16.46	
t4.45	2,2′,3,4′,5,5′,6-heptaCB (PCB187)	20	14	Middle	1.27	0.49-3.31		2.99	1.35-18.96		23	17	1.11	0.43-2.87	3.07	0.81-13.00	
t4.46		14	20	Highest	0.62	0.24-1.62		3.26	0.80-12.47		12	19	0.52	0.19-1.43	3.68	0.71-21.89	
t4.47	2,2′,3,3′,4,4′,5,5′-octaCB (PCB194)	17	11	Middle	1.26	0.47-3.37		3.64	0.93-16.27	0.060	19	10	1.55	0.58-4.17	3.85	1.00-17.04	0.051
t4.48		13	21	Highest	0.51	0.20-1.29		3.45	0.61-22.50		11	22	0.41	0.16-1.06	2.86	0.48-19.26	
t4.49	2,2′,3,3′,4,5,6,6′-octaCB (PCB199)	20	13	Middle	1.23	0.47-3.21		3.04	0.83-12.59		19	11	1.43	0.54-3.72	3.89	1.00-16.80	0.054
t4.50		12	21	Highest	0.46	0.17-1.20		2.04	0.43-10.71		11	21	0.43	0.16-1.12	2.31	0.48-12.61	
t4.51	2,2′,3,3′,4,4′,5′,6-octaCB and 2,2′,3,	23	11	Middle	2.09	0.78-5.59		**7.50**	1.72-40.29	0.004	22	13	1.41	0.53-3.72	**4.19**	1.07-19.09	0.038
t4.52	4, 4′,5,5′,6-octaCB (PCB196-203)																
t4.53		12	22	Highest	0.54	0.21-1.44		4.67	0.78-33.23		12	22	0.45	0.17-1.21	3.28	0.56-21.38	
t4.54	**Persistent Pesticides**																
t4.55	Hexachlorobenzene (HCB)	18	15	Middle	1.27	0.49-3.30		3.55	0.90-16.35		15	17	0.63	0.24-1.64	1.06	0.29-3.99	
t4.56		17	17	Highest	1.06	0.41-2.72		2.46	0.64-10.29		16	18	0.63	0.25-1.63	1.58	0.43-6.38	
t4.57	β-Hexachlorocyclohexane	20	15	Middle	2.05	0.78-5.39		3.87	0.96-18.35		22	11	2.02	0.75-5.42	3.79	0.98-17.20	
t4.58	(B-HCCH)																
t4.59		16	18	Highest	1.13	0.43-2.93		2.71	0.72-11.36		13	20	0.74	0.29-1.92	2.13	0.57-8.63	
t4.60	Oxychlordane	18	16	Middle	0.89	0.34-2.31		2.16	0.59-8.60		19	14	1.00	0.37-2.65	3.21	0.81-14.64	
t4.61		15	19	Highest	0.62	0.24-1.62		2.61	0.55-14.25		14	20	0.52	0.20-1.36	2.21	0.47-11.56	
t4.62	Trans-Nonachlor	18	16	Middle	1.00	0.39-2.59		2.19	0.60-8.67		18	16	0.89	0.34-2.31	2.17	0.58-8.80	
t4.63		16	18	Highest	0.79	0.30-2.05		3.16	0.64-18.15		15	19	0.62	0.24-1.62	1.95	0.43-9.67	
t4.64	2,2-Bis(4-chlorophenyl)-1,	21	13	Middle	1.61	0.62-4.24		2.35	0.67-8.74		21	13	1.44	0.55-3.77	2.93	0.78-12.30	
t4.65	1–dichloroethene (DDE)	14	20	Highest	0.70	0.27-1.82		1.15	0.31-4.37		13	21	0.55	0.21-1.44	1.14	0.30-4.44	
t4.66	2,2-Bis(4-chlorophenyl-1, 1,	13	20	Middle	0.52	0.20-1.36		0.45	0.12-1.62		15	18	0.62	0.24-1.63	0.79	0.22-2.79	
t4.67	1-trichloroethane (DDT)	19	14	Highest	1.09	0.42-2.82		1.29	0.35-4.84		17	16	0.80	0.31-2.07	1.02	0.30-3.52	
t4.68	**Perfluorinated compounds**																
t4.69	PFOA	15	18	Middle	2.32	0.83-6.45		1.65	0.45-6.14								
t4.70	Perfluorooctanoate	28	7	Highest	**11.11**	3.61-34.24	0.000	**6.93**	1.79-29.92	0.003							
t4.71	PFOS	19	13	Middle	**3.80**	1.38-10.48	0.010	3.43	0.95-13.31	0.062							
t4.72	Perfluorooctane sulfonate	23	11	Highest	**5.44**	1.95-15.13	0.001	**5.79**	1.58-24.12	0.005							

Table 4 Logistic regression for middle and highest tertile serum concentrations compared to the lowest tertile for brominated diphenyl ethers, polychlorinated biphenyls, organochlorine pesticides, and perfluorinated compounds *(Continued)*

t4.73	PFHxS	16	12	Middle	2.00	0.75-5.33	0.85	0.20-3.31
t4.74	Perfluorohexane sulfonate	20	14	Highest	2.14	0.84-5.44	1.20	0.35-4.07
t4.75	PFNA	17	13	Middle	2.15	0.80-5.73	1.13	0.37-4.49
t4.76	Perfluorononanoate	21	14	Highest	2.46	0.95-6.36	2.25	0.67-8.00

Q1 t4.77 [a]Controlling for age, BMI, and race.

Table 5 Urinary concentrations of phthalate metabolites and bisphenol A in polycystic ovary syndrome (PCOS) case-patients and controls

Agents		Geometric mean concentration (μg/L)		Creatinine-adjusted geometric mean concentration (μg/g creatinine)	
Phthalates	**Phthalate metabolites measured**	**PCOS case-patients**	**PCOS control-patients**	**PCOS case-patients**	**PCOS control-patients**
Butylbenzyl phthalate (BBzP)	Monobenzyl phthalate (mBzP)	4.7	9.0	7.5	11.7[a]
Di-n-butyl phthalate (DBP)	Mono-n-butyl phthalate (mBP)	15.3	24.8	17.7	23.2
Diethyl phthalate (DEP)	Monoethyl phthalate (mEP)	103.7	138.3	181.1	195.8
Di-isodecyl phthalate (DiDP)	Mono(carboxynonyl) phthalate (mCNP)	3.2	3.0	3.6	2.6
Di-isononyl phthalate (DiNP)	Mono(carboxyoctyl) phthalate (mCOP)	6.7	9.0	7.8	8.4
Di-n-octyl phthalate (DOP)	Mono-3-carboxypropyl phthalate (mCPP)	2.4	3.6	2.8	3.4
Di-2-ethylhexyl phthalate (DEHP)	Mono-2-ethyl-5-carboxypentyl phthalate (mECPP)	41.5	43.2	47.9	40.4
	Mono-2-ethyl-5-hydroxyhexyl phthalate (mEHHP)	23.7	28.3	27.3	26.4
	Mono-2-ethylhexyl phthalate (mEHP)	3.3	4.0	3.2	3.5
	Mono-2-ethyl-5-oxohexyl phthalate (mEOHP)	14.0	17.4	16.2	16.3
Di-isobutyl phthalate (DiBP)	Mono-isobutyl phthalate (miBP)	6.0	8.7	7.0	8.2
Phenols					
bisphenol A (BPA)		1.6	2.1	1.6	1.9

[a]$p = 0.02$ where controls have higher mBzP urinary concentrations than case-patients.

documented evidence of an association between PCOS and serum concentrations of PFCs or PCBs.

PCOS case-patients had urinary mBzP concentration lower than controls, and *lower* concentrations of mBzP, mBP, mEHP and mEP were associated with an increased likelihood of PCOS. Previous research suggests that some phthalates, including di-2-ethylhexyl phthalate (DEHP), the precursor of mEHP, have anti-androgenic effects in animals [12]. Also, epidemiologic studies have linked mEHP and a metabolite of di-isononyl phthalate (DiNP) with decreased testosterone production in men [31] and mBP, mono-isobutyl phthalate, mBzP, and the sum of metabolites of DEHP and of DiNP with delayed pubarche in women [32]. PCOS is characterized by hyperandrogenemia; therefore, our results are consistent with previous evidence of the anti-androgenic effects of certain phthalates.

Unlike previous studies [33-35], we found no association between PCOS and BPA. Previous investigators quantified serum BPA concentrations using a commercially available enzyme-linked immunosorbent assay (ELISA) kit, whereas we measured urinary concentrations of this toxicant using the gold-standard detection technique, isotope dilution mass spectrometry [20]. ELISA lacks adequate analytical selectivity and specificity, and because matrix effects may induce performance anomalies, ELISA is not adequate for the quantitative determination of BPA in clinical specimens [34,36].

Limitations

This study is subject to several limitations. First, our sample size of 52 PCOS case-patients and 50 controls may not be large enough to generate enough statistical power to detect a difference in some of the toxicant concentrations among PCOS case-patients and controls. Similarly, the small sample size limits the possibility of considering non-monotonic dose–response relationships and some significant associations can be chance findings due to the large number of statistical tests.

We obtained a single spot serum and urine specimen. Therefore, the concentrations of each toxicant represent a snapshot of each woman's exposure at a given time. A single measurement design may be ineffective in detecting an association between PCOS and pollutants that metabolize quickly. For example, some of the measured analytes (such as PCBs, PBDEs, OCPs, and PFCs) persist in the body for years, while phthalates and BPA metabolize quickly and are eliminated from urine within a few hours after exposure.

It is unclear whether the observed associations suggest that PFOA, PFOS, and the implicated PCB congeners increase risk for PCOS or whether the endocrine milieu of the disorder alters the storage and clearance of these chemicals, leading to increased serum measurements in PCOS patients. Oligomenorrhea and amenorrhea are common symptoms of PCOS. Thus, PCOS cases, who menstruate less frequently compared

t6.1 **Table 6 Logistic regression for middle and highest tertile creatinine-adjusted urine concentrations compared to the**
t6.2 **lowest tertile for phthalate metabolites and bisphenol A**

t6.3 Agent	N Cases	N Controls	Tertile	Odds ratio	95% C.I.		Adjusted OR[a]	Exact 95% C.I	Exact p-value
t6.4 **Phthalate metabolites**									
t6.5 mBzP	16	18	Middle	0.43	0.16-1.14		**0.24**	0.06-0.86	0.025
t6.6 Monobenzyl phthalate	13	20	Highest	**0.27**	0.10-0.75	0.012	**0.15**	0.03-0.58	0.004
t6.7 mBP	15	19	Middle	**0.21**	0.07-0.58	0.012	**0.14**	0.03-0.54	0.002
t6.8 Mono-n-butyl phthalate	14	19	Highest	**0.41**	0.15-1.12	0.003	**0.25**	0.06-0.96	0.042
t6.9 mEHP	11	20	Middle	**0.22**	0.08-0.65	0.006	**0.17**	0.04-0.63	0.005
t6.10 Mono-2-ethylhexyl phthalate	16	17	Highest	0.87	0.34-2.21		0.91	0.26-3.19	
t6.11 mEP	16	18	Middle	**0.27**	0.10-0.75	0.012	**0.12**	0.02-0.48	0.001
t6.12 Monoethyl phthalate	15	18	Highest	0.66	0.25-1.75		0.30	0.06-1.18	
t6.13 mCNP	16	19	Middle	1.00	0.39-2.59		0.80	0.23-2.71	
t6.14 Mono (carboxynonyl) phthalate	18	14	Highest	1.06	0.41-2.77		1.45	0.43-5.07	
t6.15 mCOP	18	16	Middle	**2.82**	1.05-7.60	0.040	2.27	0.61-8.84	
t6.16 Mono (carboxyoctyl) phthalate	14	18	Highest	1.45	0.55-3.85		1.58	0.46-5.55	
t6.17 mCPP	16	14	Middle	0.78	0.30-2.07		0.83	0.23-2.91	
t6.18 Mono-3-carboxypropyl phthalate	14	19	Highest	0.54	0.21-1.44		0.36	0.09-1.28	
t6.19 mECPP	16	17	Middle	0.95	0.36-2.45		0.78	0.22-2.73	
t6.20 Mono-2-ethyl-5-carboxypentyl	16	17	Highest	1.85	0.69-4.91		2.09	0.57-8.16	
t6.21 mEHHP	15	19	Middle	0.66	0.25-1.72		0.50	0.15-1.66	
t6.22 Mono-2-ethyl-5-hydroxyhexyl	16	17	Highest	1.00	0.38-2.63		1.27	0.34-4.96	
t6.23 mEOHP	16	17	Middle	0.94	0.36-2.45		0.88	0.27-2.87	
t6.24 Mono-2-ethyl-5-oxohexyl phthalate	15	18	Highest	0.84	0.32-2.18		1.07	0.29-4.00	
t6.25 miBP	17	17	Middle	0.58	0.22-1.54		0.56	0.16-1.91	
t6.26 Mono-isobutyl phthalate	12	21	Highest	0.40	0.15-1.08		0.31	0.08-1.08	
t6.27 **Phenols**									
t6.28 BPA	15	16	Middle	0.43	0.16-1.15		0.44	0.13-1.46	
t6.29 Bisphenol A	14	20	Highest	0.84	0.32-2.21		0.73	0.20-2.58	

t6.30 [a]Controlling for age, BMI, and race.

to controls, may have similar exposures to women without PCOS, but higher blood-toxicant concentrations [23]. PCBs, however, are stored mostly in adipose tissue making amenorrhea an unlikely explanation for differences in measured exposure.

Finally, a larger proportion of case-patients were of White race compared to controls, and PCOS patients were younger and had significantly higher BMI than controls, although we attempted to control for these parameters known to be associated with case-status in the logistic regression models.

Conclusions

In summary, associations between PCOS and serum concentrations of PFOS and PFOA as well as some PCBs were observed in this pilot study. This research highlights the need to further substantiate the association between PCOS and exposure to these pollutants using different study designs such as including large cohorts and measuring for additional confounders such as adiposity. The relationships between environmental contaminants are complex. Humans are exposed to many endocrine disrupting chemicals at once. Some chemicals have androgenic effects while others have anti-androgenic effects and for some chemicals those effects are indirect, rather than being a direct agonist or antagonist against specific hormone receptors. It is, therefore, difficult to evaluate true associations between environmental contaminants and disease. Thus, further studies are needed to explore the potential mechanisms by which these chemicals might contribute to the development of PCOS.

Additional files

Additional file 1: Limits of Detection (LODs).

Additional file 2: Fifty percent or more of the samples examined had concentrations below the limit of detection (LOD) for these chemicals.

Additional file 3: Correlations of brominated diphenyl ethers (BDE), polychlorinated biphenyls (PCB), organochlorine pesticides (OCP), perfluorinated compounds (PFC), phthalates, and bisphenol A.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SV analyzed the data, interpreted the results and drafted the manuscript, EAZ contributed to the conception and design of the study, interpreted results of the data analysis and assisted in drafting the manuscript. AS and AC analyzed data and contributed to drafting the manuscript. DD and LG provided interpretation of data analyses and critical review of the manuscript. KK MS and XY analyzed data, provided interpretation of data analyses and critical review of the manuscript, and RA designed the study, oversaw data collection, interpreted results, and provided critical review of the manuscript. All authors read and approved the final manuscript.

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