HHS Public Access

Author manuscript

Environ Res. Author manuscript; available in PMC 2019 July 01.

Published in final edited form as:

Environ Res. 2018 July ; 164: 556–564. doi:10.1016/j.envres.2018.03.021.

Persistent organic pollutants as predictors of increased FSH:LH ratio in naturally cycling, reproductive age women

Mia V. Gallo[1,2,3,*], **Julia Ravenscroft**[1], **David O. Carpenter**[2,3], **Lawrence M. Schell**[1,2,3,4], and **Akwesasne Task Force on the Environment**[4]

[1]University at Albany, Department of Anthropology, A&S 237, 1400 Washington Avenue, Albany, NY

[2]Center for the Elimination of Minority Health Disparities, University at Albany-SUNY; 1400 Washington Avenue, Albany, NY

[3]Institute for Health and the Environment, University at Albany, 5 University Place, Rensselaer, NY

[4]University at Albany, Department of Epidemiology and Biostatistics, School of Public Health, One University Place, Room 131, Rensselaer, NY

[5]Akwesasne Task Force on the Environment, Akwesasne Mohawk Nation, Akwesasne, NY

Abstract

Although several recent studies suggest endocrine disrupting compounds, such as polychlorinated biphenyls (PCBs), dichlorodiphenyldichloroethylene (*p,p′*, DDE), and hexachlorobenzene (HCB), target different organs and systems in the body, their impact on female reproductive function in humans is not well characterized. We seek to determine the relationship between several known endocrine disrupting compounds and a marker of ovarian responsivity, the FSH:LH ratio (higher ratio indicates less ovarian responsivity).

For this analysis, 169 naturally cycling women between 21 and 38 years of age completed interviews and had their blood drawn on day 3 of their menstrual cycle for analyses of toxicants, gonadal sex hormones (E2 and P4), and gonadotropins (FSH and LH). PCB congeners were classified into five groups based on their environmental persistence, distribution in human tissue, and toxicological action, reflecting the structure, mechanism, and known biological activity of individual PCB congeners.

[*]Corresponding Author: Mia V. Gallo, A&S 237, University at Albany, 1400 Washington Ave., Albany, NY 12222. Telephone: 518-442-4720; Fax: 518-442-4563. mvgallo@albany.edu.

Conflict of interest

Dr. Carpenter has served as an expert witness in legal actions against the General Motors Corporation with all reimbursements deposited into a Research Foundation account with the University at Albany. The Mohawk Nation at Akwesasne has also been party to legal actions related to contamination within the reserve. All other co-authors declare they have no actual or potential competing financial interests.

For every unit (ppb) increase in the level of the estrogenic PCB group, there was a 5 fold greater risk of a FSH:LH ratio ≥ 2, controlling for individual differences in age, percent body fat, cycle day 3 estradiol levels, parity, alcohol use and cigarette smoking in the past year (exp[β] = 5; p = ≤0.01). PCB congeners identified as estrogenic were analyzed individually, and, of the 19 potentially estrogenic congeners, five were significantly, and positively related to an increased FSH:LH ratio. Four of these congeners are non-persistent, easily volatilize in the environment, and are easily metabolized, and hence, are indicative of very recent or current exposure. *p,p′*-DDE and HCB were not associated with FSH:LH ratio.

We find a clinical indicator of ovarian responsivity, FSH:LH ratio, is associated with a specific group of estrogenic PCBs. These congeners may become airborne when they volatilize from dredged PCB-contaminated soil or from indoor PCB-containing window caulk and sealants in older buildings leading to inhalation exposure. PCB exposure, particularly to non-persistent, estrogenic congeners, may pose an unrecognized threat to female fecundity within the general population.

Keywords

Polychlorinated biphenyls; PCBs; *p,p′*-DDE; HCB; endocrine disrupting chemicals; persistent organic pollutants; follicle stimulating hormone; luteinizing hormone; FSH; LH; FSH:LH ratio; ovarian responsivity; Mohawk; Native American

1. Introduction

Reproductive health implies in part the capability to reproduce. A woman's reproductive system is highly complex, and female factor infertility can be the result of age, hormonal dysfunction, lifestyle or environmental factors. Approximately 15% of women in the US between 15 and 44 years of age suffer from impaired fecundity, and 6% are considered infertile (https://www.cdc.gov/nchs/fastats/infertility.htm). Between 2006 and 2010, 7.3 million women (or 12%) used infertility services (Chandra et al. 2014), and in 2013 a total of 160,521 assisted reproductive technology (ART) procedures were performed (Sunderam et al. 2015). Despite this growing use of technology, costs associated with ART can be overwhelming. As such, given the burden of infertility both at an emotional level and financially, understanding factors that can perturb the menstrual cycle by delaying or preventing conception is important.

Organochlorines such as polychlorinated biphenyls (PCBs), act as endocrine disrupting chemicals (EDCs), and interfere with normal endocrine homeostasis, impact female fecundity, as well as alter menstrual cycle characteristics including ovulation (Buck Louis et al. 2006; Gallo et al. 2016; Mendola et al. 1997; Sallmen et al. 2005; Toft et al. 2004; Toft 2014; Karwacka et al. 2017). Data from the 2001–2002 NHANES study of women of childbearing age show that 80% of participants had detectable levels of PCBs (Axelrad et al. 2009). Low concentrations of EDCs may disrupt endocrine regulated processes necessary for normal function of the female reproductive system, including the ovarian cycle, suggesting the potential to disturb fecundity (Germaine M. Buck et al. 1997; G. M. Buck et al. 1997; Gallo et al. 2016; Mendola et al. 1995; Mendola et al. 1997; Windham et al. 2002;

Yu et al. 2000; Karwacka et al. 2017). However, the effects of this ubiquitous pollutant on gonadotropins necessary for successful conception has not been examined.

Although organochlorine exposure in non-occupationally exposed populations is often largely attributed to dietary pathways, there is increasing evidence that inhalation of some organochlorines, especially airborne polychlorinated biphenyls, constitutes as great an exposure as the dietary route in some populations and has the potential for adverse health effects (Lehmann et al. 2015). Construction materials such as caulks and sealants often contain commercial PCB mixtures, and the more lightly chlorinated PCB congeners found in these products can volatize producing chronic airborne exposure (Dumanoglu et al. 2016; Frederiksen et al. 2012; Hu et al. 2012; Melymuk et al. 2016; Persoon et al. 2010). As such,

Normally gonadotrophin-releasing hormone (GnRH) stimulates the anterior pituitary gonadotropes to secrete the two key hormones, follicle stimulating hormone (FSH) and luteinizing hormone (LH), which act synergistically in a pulsatile manner (Kronenberg and Williams 2008). In the early follicular phase of a menstrual cycle, FSH secretion increases slightly stimulating follicular recruitment and emergence of the dominant follicle. In response to FSH, granulosa cells in the follicles produce estradiol (E2) stimulating further production of GnRH and downstream LH with concurrent slowing of FSH as the dominant follicle transitions from FSH to LH dependence (Son at al., 2011). During the last half of the follicular phase E2 levels continue to rise followed by an LH surge which triggers ovulation. Within clinical settings, menstrual cycle day 3 levels of FSH, LH, and E2 along with chronological age are often considered baseline markers of potential ovarian responsivity and oocyte quality (Gougeon et al. 1994; Seckin et al. 2012).

Elevated cycle day 3 FSH levels are associated with decreased oocyte quality, quantity, and fertility response (Abdalla and Thum 2004; Broekmans et al. 2009). Additionally, poor ovarian response to gonadotrophin stimulation in women undergoing ART is associated with a high day 3 FSH:LH ratio, a biomarker of ovarian responsivity, even with a day 3 FSH level within the normal reference range (Barroso et al. 2001; Broekmans et al. 2006; Liu and Greenblatt 2008; Prasad et al. 2013). As nearly 10% of reproductive-age women experience a faster than expected decline in fertility (Nikolaou et al. 2009), elucidating the determinants of ovarian responsivity above and beyond maternal age is essential.

Here we examine the association of specific persistent organochlorine pollutants on cycle day 3 FSH, LH, and E2 levels in a sample of women with known exposure due to local environmental pollution. Furthermore, we investigate the PCB congeners that are most associated with an altered FSH:LH ratio, which then provides important information as to the route of exposure to the PCBs, since the lower chlorinated congeners are more volatile and will be found in air (Lehmann et al. 2015; Melymuk et al. 2016), whereas the more highly chlorinated congeners are those found in food, such as contaminated fish (ATSDR 2000, 2011; Forti et al. 1995).

2. Methods

2.1. Sample and site characteristics

Women in this study were drawn from the population of Native Americans from the Akwesasne Mohawk Nation (AMN). The AMN is a sovereign nation situated on the St. Lawrence River with territory bordering New York State, Ontario and Quebec, Canada. The Nation is in close proximity to three industrial sites, specifically the General Motors Central Foundry Division, Reynolds Metal Company and Aluminum Company of America (Sloan and Jock 1990; Lacetti 1993), known to have discharged significant quantities of PCBs (primarily Aroclor 1248) into the St. Lawrence River and its three tributaries. One became a National Priority Superfund Site and the two others New York State Superfund Sites (New York State Department of Environmental Conservation 2000). Local species of fish, birds, amphibians and mammals had toxicant levels exceeding the US Food and Drug administration's tolerance limits for human consumption (Forti et al. 1995; Sloan and Jock 1990). As part of a traditional Native American diet, the consumption of fish and wildlife was usual until advisories were issued in the mid-1980's (Fitzgerald et al. 1995; Fitzgerald et al. 1998; Fitzgerald et al. 2004).

In the context of known PCB exposure and to address concerns of the Akwesasne Mohawk community regarding women's fertility, we recruited 215 women between 2009 and 2013, to assess the relationship between environmental toxicants and reproductive function. To be eligible for the project, women were required to be Akwesasne residents and between 21 and 38 years of age. A woman was ineligible if she was: 1) taking any form of hormonal birth control; 2) pregnant or nursing (though could reapply in 6 months' time); and 3) taking any medications for thyroid dysfunction.

Sample characteristics, recruitment, and attrition have been described previously (Gallo et al, 2016). In brief, 215 women enrolled in the project. Of these, 31 women decided against completing the full requirements of the project which included salivary collection over the course of one menstrual cycle. Of the remaining 184 women, 15 women enrolled in the project with a history of menstrual irregularity and were considered non-cycling women (NCW). They were not included in this analysis, hence reducing the sample to a final size of 169 participants. These 169 women completed interviews and had their blood drawn for toxicant, gonadal sex hormones (E2 and P4), and gonadotropin (FSH and LH) analysis on day 3 of their menstrual cycle. With the exception of the non-cycling women, none of the participants in this analysis had any fertility concerns as assessed from their reproductive history questionnaire, a medical diagnosis of reproductive dysfunction (PCOS or endometriosis), or were actively trying to conceive at the time of enrollment.

2.1.a. Ethical Approval—All data were collected by project staff who were members of the Akwesasne community; all were without prior knowledge of participants' exposure status. The University at Albany's Institutional Review Board approved all study protocols and informed consent procedures.

2.2 Data collection

Interview and questionnaires obtained information on sociodemographic and behavioral characteristics (i.e. marital status, educational attainment, smoking and alcohol consumption, physical activity, religious and spiritual affiliation, residential and occupational history, and material well-being), dietary (24-hour recall, and consumption of locally grown, hunted, trapped, or fished foods), and reproductive histories (i.e. parity, gravidity, menstrual cycle length).

Anthropometric measurements (including height, weight, seven skinfolds, three breadths, and five circumferences) were measured according to standard protocols (Lohman et al., 1988) and taken in triplicate by two trained data collectors. The principal investigators (LMS, MVG) conducted measurement training, and retraining occurred at 6-month intervals. All women were measured in standard, very lightweight, non-restrictive clothing (disposable shorts and a tee shirt). Standing height was measured using a wall-mounted stadiometer (Seca 216 ©, Hamburg, Germany) to the nearest millimeter. Weight was measured with an electronic scale (Tanita 2009©; Tokyo, Japan). Skinfolds were measured with a Lange skinfold caliper (Cambridge Scientific Industries, Cambridge, MD) to the nearest 0.5mm. The calipers were checked repeatedly for calibration with the manufacturer's calibration block. Body mass index (BMI; weight (kg)/height $(m)^2$) was calculated for each woman and classified into adult weight status categories (normal weight, overweight and obese; http://www.cdc.gov/healthyweight/assessing/bmi/adult_bmi/index.html). Since BMI has been established as a poor predictor of body fat among different ethnic groups, age-specific body density was calculated according to a well validated and standard formula using the sum of four skinfolds (Durnin and Womersley,1974) and the Siri (1956) equation was then applied to calculate percent body fat (Arroyo et al., 2004; Deurenberg et al. 1991, 1998).

2.3 Clinical chemistries and toxicant analyzes

Participants' blood was drawn on Day 3 of their cycle for analysis of lipid levels (total cholesterol, triglycerides), serum estradiol (E_2; reference range 12.5–166.0 pg/mL for follicular phase), progesterone (P_4; reference range 0.2–1.5 ng/mL for follicular phase), follicle stimulating hormone (FSH; (reference range 3.5–12.5 mIU/mL for follicular phase), and luteinizing hormone (LH; reference range 2.4–12.6 Follicular mIU/mL for follicular phase). All assays were conducted by LabCorps, Inc. using lectrochemiluminescence immunoassay (ECLIA).

The University at Albany's Exposure Assessment Laboratory conducted all organochlorine pesticide and PCB analyses for this project. This laboratory is accredited by the NYSDOH Clinical Laboratory Evaluation Program and participates in the Arctic Monitoring and Assessment Programme Ring Test for Persistent Organic Pollutants in Human Serum. Blood specimens provided material for analysis of four toxicants (101 individual PCB congeners, p,p'-DDE, HCB, and mirex). Analysis of low and high-level QC performance samples indicated accuracy and precision of ±15% of nominal and ≤15% RSD, respectively. Complete details of the laboratory protocol for PCB analysis have been published (DeCaprio et al. 2000, 2005). In brief, high resolution, ultratrace, congener-specific analysis was

performed by parallel dual-column (splitless injection) gas chromatography (GC) with electron capture detection (ECD) on an Agilent instrument. This method quantitates up to 83 individual PCB congeners and 18 PCB congeners as pairs or triplets (a total of 94 analytical peaks), including HCB, *p,p′*-DDE, and mirex. The analytes include most of the major Aroclor-derived congeners typically present in human samples plus a number of sporadic or rare congeners, but does not detect some of the more potent dioxin-like congeners. Data were expressed on a whole-weight basis (i.e., not lipid-adjusted) with serum lipids treated as a covariant. Individual chlorinated biphenyl (CB) congeners are referenced according to the IUPAC numbering system (Ballschmiter and Zell 1980; Guitart et al. 1993). PCB congeners for which all reported values were below the laboratory method detection limit (MDL) included CBs 1, 3, 4, 9, 10, 13, 15, 19, 29, and 40. These congeners were not included in any calculations. PCB concentrations below the MDL were assigned a value equal to the MDL divided by the square root of 2 for calculation of geometric means.

2.4. Congener groupings

Eighteen PCB congeners were detected in 50% or more of the sample (CBs: 31, 52, 66, 70, 74, 84, 87, 101, 105, 110, 118, 136, 138, 153, 158, 180, 183, 187). An additional 30 PCB congeners were detected in 20% or more of the sample (CBs: 28, 32, 33, 42, 44, 47, 49, 51, 53, 56, 63, 64, 71, 83, 91, 92, 95, 99, 114, 128, 144, 151, 156, 170, 172, 174, 177, 185, 190, 199).

Given their environmental persistence, distribution in human tissue and toxicological action, the composition of PCB groupings for analytical purposes was contingent on both the structure, mechanism, and known biological activity of individual PCB congeners (Brown 1994; Hansen 1999; Laden et al. 1999; McFarland and Clarke 1989; Safe 1984, 1995, 2001, 2004). Therefore, we grouped PCB congeners detected in 20% or more of the sample by the number of *ortho*-substituted chlorines:

- Non-/mono-*ortho* biphenyls (ΣMonoOrthoPCBs): CBs: 28, 31, 33, 56, 63, 66, 70, 74, 105, 114, 118, and 156;
- Di-*ortho* biphenyls (ΣDiOrthoPCBs): CBs: 32[16], 42, 44, 47[59], 49, 52, 64, 71, 83, 87, 92, 99, 101[90], 110, 128, 138[164+163], 153, 158, 170, 172, 180, and 190;
- Tri-/tetra-*ortho* biphenyls (ΣTriTetraOrthoPCBs): CBs: 51, 53, 84, 91, 95, 136, 144, 151, 174, 177, 183, 185, 187, 199, and 203.

Also, many reports have detected associations between certain PCB congeners and estrogenic activity (Cooke et al. 2001, Pliskova et al. 2005, Pollack et al. 2011, and Wolff et al. 1997). However, the congeners identified as estrogenic are not the same across studies with only CB52 in common. To reduce error which could occur through repeated analysis of slightly different estrogenic groupings, and since each of these research teams considered different PCB congeners to be estrogenic or mildly estrogenic, we combined their 19 specified PCB congeners into one group (ΣEstrogenicPCBs; CBs: 28, 31, 44, 47[59], 49, 52, 66, 70, 74, 95, 99, 101, 105, 110, 136, 153, 174, 177, and 187 (Cooke et al. 2001; Pliskova et al. 2005; Pollack et al. 2011; Wolff et al. 1997). Within the estrogenic group of PCBs

there are four that are frequently measured in air due to their high volatility. We have grouped these congeners together also (ΣAirBornePCBs; CBs: 28, 52, 66, 74).

Two endocrine disruptive organochlorine pesticides, dichlorodiphenyldichloroethylene (*p,p′*-DDE, a stable metabolite of DDT), and hexachlorobenzene (HCB) detected in 100% and 97% of the sample respectively were also included in the analysis to examine effects of concomitant exposure. Mirex, at a rate of detection less than 5%, was not considered in this analysis.

We examined cycle day 3 FSH:LH ratio, as a proxy for ovarian responsivity, in relation to levels of persistent organic pollutants (POPs). FSH:LH ratio of ≥ 2 was considered elevated (Barosso et al., 2001; Liu and Greenblatt, 2008; Lyu et al., 2013; Prasad et al., 2013; Shrim et al., 2006).

2.5. Statistical analysis

Statistical analyses were conducted using SPSS v.23 (IBM, 2015). Two multivariate approaches were employed for this analysis. In the first approach, we applied logistic regression to estimate associations of DOR/POI with the five PCB groupings, *p,p′*-DDE and HCB levels individually (only one toxicant per model; Model 1) including confounders. If an association was observed with a particular PCB grouping, we then tested the individual congeners separately within the grouping to assess significance at $\alpha \leq 0.05$. To address concomitant exposure, the second approach included both *p,p′*-DDE and HCB in the model with each PCB grouping or congener (Model 2). PCBs, individually and in groups, as well as the other toxicants were modeled as continuous variables (log-transformed).

We considered factors as potentially confounding and suitable for inclusion in our models based on previous research suggesting associations with ovarian responsivity. Potential confounding factors were evaluated by either t-test or bivariate correlation at p=0.10. Age, percent body fat (%BF), cycle day 3 estradiol (E2) levels, parity, alcohol use in the past year (yes/no), and cigarette smoking in the past year (yes/no) met the criteria for confounding and were included in all adjusted models. Since PCBs are lipophilic and localized in serum lipids, lipids were included in the model as covariates as this approach is preferable to using whole weight toxicant levels (PCB, HCB, or *p,p′*-DDE per unit of serum) as it creates bias (Phillips et al., 1989; Schisterman et al., 2005). Total lipids were estimated by the formula first described by Phillips et al (1989) and later updated by Bernert et al. (2007):

$$Total\ lipids\,(mg/dL) = (2.27 * cholesterol\ level) + (Triglycerides) + 0.623 mg/dL\,.$$

3. Results

Mean age of the sample was 30.3 years. Forty percent of the women were nulliparous, and 48.5% had never been married but 51.5% lived with a significant other. Using CDC criteria, 57% of the women were obese, and 23% considered overweight (Table I).

Of the 169 women included in this analysis only 10% (18) considered their menstrual cycle ‘irregular’. Twelve women had gonadotropins outside the reference range for the follicular

phase: one woman had elevated E2 and P4 levels, six women had elevated P4 levels, one an elevated FSH level, and another four had elevated LH levels (Table II). Women's age was positively associated with FSH levels, while LH levels were negatively related to age. Percent body fat and BMI were inversely related to LH, yet positive in relation to FSH:LH ratio. Twenty-one women (12%) had a FSH:LH ratio ≥ 2. These women did not differ from those with an FSH:LH ratio < 2 in terms of age, height, total lipids, cholesterol, triglycerides, marital status, cigarette or alcohol use, FSH, E2, or P4 levels. However, they were significantly heavier (median 94.9 vs 82.3 kg), had a greater BMI (median 34.2 vs 30.7 kg/m^2), and a higher percent body fat (41.4% vs 38.9%) than those with an FSH:LH ratio <2. These women also had lower mean LH levels (median 2.7 vs. 5.4 mIU/mL). Women in the youngest age category (21–24.99, n=30) had significantly higher LH levels than women in older age categories (p=0.03).

Only levels of ΣEstrogenicPCBs and ΣTriTetraOrthoPCBs increased with age (r^2 = 0.19 and 0.24 respectively). Mean levels of ΣMonoOrthoPCBs were moderately, yet significantly higher in obese women in comparison to overweight women, but not to women of normal weight (p=0.05). Cycle day 3 FSH and LH levels were not significantly related to any of the PCB groupings or other organochlorines (*p,p,*-DDE and HCB). Mean levels of four different PCB groupings were significantly higher in women with a FSH:LH ratio ≥ 2 (Table III), while the two groups of women did not differ in levels of *p,p′*-DDE or HCB.

In single toxicant models (Model 1), total lipids, cigarette use in the past year, and parity were not significant predictors of a FSH:LH ratio >2. Alcohol consumption in the past year was suggestive but not significant (p=0.71–0.11) with the exception of the model that included ΣMonoOrthoPCBs (β =1.5; p=0.04). However, both estradiol levels (β = −0.66 through −0.77; p = ≤0.01 to 0.02), and %BF (β = 0.16–0.17; p = 0.02–0.04) were consistently and significantly related to FSH:LH ratio in all single toxicant models. For every unit decrease in E_2, there was a likelihood of a decrease in FSH:LH ratio (EXP[β] = 0.9) and conversely, for every unit increase in percent body fat there was a 20% greater likelihood of a FSH:LH ratio > 2 (EXP[β]=1.2).

Controlling for individual differences in age, %BF, cycle day 3 E_2 serum levels, parity, alcohol use and cigarette smoking in the past year, the dichotomous FSH:LH ratio was not related to either *p,p′*-DDE or HCB (β=0.47, p= 0.31; β=0.76; p=0.23 respectively). A significant positive association was observed with four of the five PCB groupings (Table IV). Increasing concentrations of ΣAirBornePCBs, ΣEstrogenicPCBs, ΣMonoOrthoPCBs, and ΣTriTetraOrthoPCBs were significantly associated with a FSH:LH ratio >2 (Table IV). The ΣDiOrthoPCB grouping was suggestive yet not significant (p=0.06; see Table IV). Using the continuous FSH:LH ratio yielded similar results. The ΣAirBornePCBs, ΣEstrogenicPCBs, and ΣMonoOrthoPCBs groupings were found to be significant predictors of the FSH:LH ratio (p = 0.02, 0.04, and 0.03 respectively); however, ΣTriTetraOrthoPCBs was not (p = 0.62).

For the purpose of interpreting these results, we will concentrate on the relationship between ΣEstrogenicPCBs, specifically because this grouping combines most of the specific PCB congeners found in ΣMonoOrthoPCBs, ΣAirBornePCBs and ΣTriTetraOrthoPCBs. For

every unit increase in levels of the ΣEstrogenicPCBs, there is a 5 times greater risk of a FSH:LH ratio ≥ 2, controlling for individual differences in age, %BF, cycle day 3 E_2 levels, parity, alcohol use and cigarette smoking in the past year (EXP[β] = 5; Figure I). In fact, just a 20% increase in levels of ΣEstrogenicPCBs, increases the odds of having a FSH:LH ratio above 2 by 37% and a 50% increase in ΣEstrogenicPCBs increases the odds by 120%. The inclusion of *p,p′*-DDE and HCB (Model 2) did not change these results.

To further assess which specific PCB congeners within the ΣEstrogenicPCB group had the potential to affect FSH:LH ratio, each CB was included separately in a single toxicant logistic model controlling for the same factors. Of the potentially estrogenic 19 congeners, five congeners with a rate of detection (ROD) > 30% were significantly, and positively related to an increased FSH:LH ratio: CBs 47[59], 52, 95, 101, and 105 (see Table IV). Three of these were detected in 50% or more of the sample CBs 52, 101, and 105. A 50% increase in either CB 52, 101, or 105 increases the odds of having a FSH:LH ratio above 2 by 45%, 51%, or 37% respectively.

4. Discussion

We found that an indicator of ovarian responsivity is statistically associated with a specific set of PCBs. Of the five specific PCB congeners identified as predictors of an increased FSH:LH ratio, only CB 105 is classified as persistent and resistant to degradation (Hansen 1999). The other four congeners (CBs 47[59], 52, 95, and 101) are non-persistent, easily metabolized, and hence are indicative of current exposure. All five congeners volatilize easily into the atmosphere and exposure through inhalation is continuous (Dumanoglu et al., 2016; Frederiksen et al. 2012; Hu et al. 2012; Melymuk et al., 2016; Persoon et al. 2010).

Obesity and age are known risk factors for disturbed HPG axis operation and ovarian function (McKinnon et al, 2016). This sample is composed of relatively young women and there were no differences in age between women grouped by FSH:LH ratio. Obesity however, is common in this sample (Table I) and women with FSH:LH ratios below 2.0 were significantly less heavy than those with ratios of 2.0 or higher (t= −3.2; p=0.001). Percent body fat, used as a proxy for overweight/obesity, and age were included as covariates in this analysis using multivariate models that detected strong and consistent relationships with specific PCBs. Further, removing either or both of these variables from the model did not change the relationships with PCBs. Relationships with HCB, or *p,p′*-DDE were not detected in any multivariate models, with or without other toxicants included.

There are no other studies on the relationship between PCBs and FSH:LH ratio in humans accessible through pub med as of March 21st, 2017. Our finding that this marker of ovarian responsivity is affected by a select group of estrogenic PCBs is important given the current rise in reproductive failure among women in the US. Relative exposure levels should be considered. Levels of the PCB congeners that were significantly related to FSH:LH ratio are high among this sample. While not as elevated as levels of POPs reported in poisoning incidents such as Yusho and Yucheng (Guo et al. 1997; Hsu et al. 2005; Mitoma et al. 2015; Yang et al. 2011), serum levels of PCBs in Akwesasne women are high in comparison to reported CDC data on US women within a similar age range (CDC 2009; Gallo et al. 2016).

Notably, levels of the three dominant CBs (52, 101, and 105) in this analysis are significantly higher than those reported by the CDC (t=6.3, 6.6, 6.1, p≤0.001, respectively), yet *p,p′*-DDE and HCB levels are significantly lower (t=−70.4, −67.0, p≤0.001, respectively).

The relationships of specific non-persistent, often airborne PCB congeners with an elevated FSH:LH ratio that we report are an important finding. Currado and Harrad (1998) and Lehmann et al. (2015) have both emphasized the need for study of inhalation as a route of exposure to PCBs. Volatization of PCBs has been reported from contaminated sediments (Bushart et al., 1998), landfills and manufacturing plants (Hermanson and Johnson, 2007) and contaminated bodies of water (Sandy et al., 2011). All are found at Akwesasne. Animal studies have demonstrated that inhalation is a very effective route of exposure (Casey et al., 1999; Hu et al., 2010). Teachers working in a PCB-contaminated school had higher concentrations of lighter PCB congeners (PCB 6-74) than referent populations (Herrick et al., 2011), indicating inhalation as a route of exposure. Ampleman et al. (2014) assessed both dietary and inhalation exposure of adolescents and their mothers, and found that while dietary exposure was best correlated with total PCBs, inhalation of lower chlorinated congeners was as high as one-third of total exposure. Our group found a relationship, in a slightly smaller sample (n=155) of Akwesasne women, between measures of anovulation and CBs 28 and 66, congeners most likely absorbed through inhalation (Gallo et al. 2016). Inhalation of volatile PCBs has also been associated with elevated risk of diabetes (Kouznetsova et al., 2007; Aminov et al., 2016) and cancer (Robertson and Ludewig, 2011; Carpenter, 2015). The role of exposure to inhaled PCBs for other health outcomes remains to be determined. However, exposure through inhalation could be traced to the presence of dredged PCB containing sediments stored onsite at the US Superfund site, to volatilization from the St. Lawrence River, or to building materials used in schools, homes, and industrial buildings built before their ban in the 1970s.

Strengths of this study include the use of menstrual cycle day 3 levels of gonadotropins. These measures are of clinical significance as lower levels, as well as a lower FSH:LH ratio, are indicators of greater conception likelihood in both assisted and natural conception cycles (Barroso et al. 2001; Broekmans et al. 2006; Liu and Greenblatt 2008; Prasad et al. 2013; Shrim et al., 2006; Lyu et al., 2013). Additionally, the measurement of more than 100 specific congeners allows groupings of congeners that are biologically and structurally meaningful, as indicated in previous studies investigating the endocrine disrupting potential of PCBs. The congeners associated with elevated FSH:LH ratio are often not measured and many studies only quantify PCB “marker” congeners (IUPAC #’s 118, 153, 180, 187) to estimate PCB levels. A “marker” PCB approach, however, overlooks exposure to non-persistent congeners and leaves a large proportion of an individual’s PCB burden (with potentially different structure-activity dynamics) invisible, as it is unmeasured. Consequently, this approach leaves open the possibility that relationships maybe present but remain undetected owing to the limited number of congeners analyzed. The increasing evidence that non-persistent congeners can exert profound effects on biological and endocrine systems make systematically assessing non-persistent congeners and identifying their specific mechanistic actions critical to understanding the overall behavior of PCBs as EDCs (Gallo et al. 2016; Kouznetsova et al., 2007; Aminov et al., 2016). The presence of

additional information on lifestyle, socio-demographic factors and a full anthropometric assessment by trained personnel is a significant strength given the known relationships between these factors and the HPG axis, particularly with overweight/obesity. Self-report of weight and height is commonly used but contains biases that could influence relationships described here (Gillum and Sempos, 2005; Tang et al. 2016).

Although several recent studies suggest EDCs are potent ovarian toxicants (Pocar et al., 2003; Kwintkiewicz et al., 2010, Gregoraszczuk et al., 2008), the full range of effects of EDCs on reproductive outcomes in humans as well as within animal models is unknown (Craig et al., 2011, Patel et al., 2015). Additionally, the biological mechanisms through which PCBs and other EDCs exert ovarian toxicity are not fully elucidated, and further basic mechanistic research is needed (Patel et al., 2015; Sifakis et al., 2017; Vabre et al., 2017). There are two primary biological pathways by which PCBs and other EDCs are likely to exert their effects on the ovary: the process of folliculogenesis, which ultimately determines follicle/oocyte health, and the process of steroidogenesis, the production of female steroid hormones (Craig et al., 2011; Sifakis et al., 2017).

To date, most studies concerning ovarian toxicity and EDC exposure have focused on the effects of EDCs on estrogen receptors, the aryl hydrocarbon receptor (AHR) and the androgen receptor (Craig et al, 2011). In particular, organochlorines bind with considerable potency to the AHR, inducing the expression of CYP1 genes that in turn metabolize E2 to inactive hydroxylated derivatives (Park et al., 2017; Tsuchiya et al., 2005). Consequently, the binding of EDCs with AHR results in antiestrogenic activity through increased metabolism and depletion of endogenous E2. Exposure of the primary, pre-antral and antral follicles within the ovary to EDCs can interfere with their development due to the inhibition of aromatase by EDCs and the associated reduced plasma E2 levels that potentially impair oocyte development (Craig et al, 2011; Sifakis et al., 2017).

When EDC’s bind with the AHR in the ovary, it may result in the loss of oocytes triggered by activation of the Bax gene and increased apoptosis, thus depleting the normal complement of oocytes (Craig et al., 2011; Hernández-Ochoa et al., 2009). Animal models have documented that exposure of rats to 2,3,7,8-tetrachlorodibenzo-p-dioxin, which chronically activates AHR, during their reproductive life is related to a dose-dependent onset of premature reproductive senescence, likely due to direct effects on the ovaries (Shi et al., 2007). Although similar studies of PCBs specifically are scarce, maternal exposure to PCBs in rats similarly decreases ovary weight in the offspring and follicular atresia (Shirota et al., 2006; Pocar et al., 2012; Vabre et al., 2017).

As the antral follicle is responsible for producing female sex steroid hormones, there are likely downstream effects on the process of steroidogenesis resulting from previous disruption to the oocyte during folliculogensis. EDCs may act directly on specific hormone-related nuclear receptors in addition to enzymes involved in steroidogenesis and the metabolism of hormones thus inducing local effects on ovarian function (Craig et al., 2011; Minegishi et al., 2003). By altering the expression, protein level, or activity of steroidogenic enzymes in the theca and granulosa cells within the ovary, EDCs can alter levels of sex steroid hormones (Kwintkiewicz and Giudice, 2009; Gregoraszczuk et al., 2008).

Hyperfunctioning of the theca and relative hypofunctioning of the granulosa cells within the ovary may result in women with relatively high circulating levels of LH, compared with FSH due to EDC-induced down regulation and insensitivity to steroid hormone feedback (Kwintkiewicz and Giudice, 2009).

The findings from our analysis are highly suggestive of a perturbation of FSH:LH ratio levels in response to PCB exposure, however whether the disturbance occurred during folliculogenesis, steroidogenesis, or both is not known. It is notable that while women in the <2 and ≥ 2 FSH:LH ratio categories do not have significantly different FSH levels, they do have significantly different LH levels on cycle day 3, with women with FSH:LH ratios <2 having significantly higher LH levels and higher E2 levels (approaching significance) than women with FSH:LH ratio levels ≥ 2. This relationship mirrors earlier observations of other EDC's that interfere with the development of antral follicles due to the inhibition of aromatase and the associated reduced plasma E2 levels arresting oocyte development (Craig et al, 2011; Sifakis et al., 2017).

There are some limitations to the present study. First, due to the cross-sectional study design the assessment of causality and assumptions of temporality are limited; the results cannot be used to draw definitive conclusions on associations between PCBs exposure and FSH:LH ratio levels. However, evidence from mechanistic studies in the animal literature suggest that PCB exposure has a pathophysiological role in the disruption of menstrual cycle function, ovarian health, and reproduction. Additionally, we have no explicit knowledge of when the women in the current study were exposed; however, the presence of nonpersistent congeners within their exposure profile is suggestive of a continuing source, perhaps via an inhalation exposure pathway, of current exposure as well as likely past exposure. The relatively low levels and narrow range of variation of HCB and DDE curtailed our ability to detect significant relationships between these persistent organic pollutants and FSH:LH ratio levels. It is possible that at higher levels either or both could exert influence on the FSH:LH ratio and we cannot rule out their potential to act as ovarian toxicants. Mechanistic work with animal models suggest mixture effects between specific PCB congeners and co-exposure to DDE have been observed for more highly chlorinated PCBs (Gregoraszczuk et al., 2008); further animal model work should be done specifically for PCB mixtures containing less chlorinated congeners. Lastly, a significant weakness is the number of women within the sample with an FSH:LH ratio ≥ 2. Clearly, the need for studies with larger sample sizes is indicated, but the results obtained in this analysis suggests they are warranted.

Conclusion

In summary, this study identified a subgroup of predominantly volatile, non-persistent PCBs which increased the probability of having a higher FSH:LH ratio regardless of age in women with known exposure to PCBs and other persistent organic pollutants. Future research is needed to determine the full impact of EDCs like PCBs on the ovary and, in particular, a focus is needed on the HPG axis as a whole to identify intermediate pathways that may be mediated by either the hypothalamus or the pituitary (Foster et al., 2008; Patel et al., 2015). Multiple organs are the targets of sex steroid hormones, those that are part of the HPG axis

and those extending far beyond the HPG axis to non-reproductive targets, which include bone, muscle, and skin (Diamanti-Kandarakis et al., 2009). Consequently, folliculogenesis and steroidogenesis are critical not only to the maintenance of fertility in adult women but also skeletal, cardiovascular, and brain health across the lifetime, underscoring the potential broad impact of optimal ovarian and HPG axis function on public health (Patel et al., 2015; Yonker et al, 2013). Accordingly, the motivation to understand the mechanisms underlying endocrine disruption and ovarian toxicity should be high, as the ramifications include preventing and ameliorating disruptions to fertility in women of reproductive age as well as disturbances to non-reproductive health in women of all ages potentially induced by EDC action on the ovary.

Acknowledgments

Funding source

The authors acknowledge support from the National Institutes of Health and specifically NIMHD – MD 003373. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center on Minority Health and Heath Disparities, or the National Institutes of Health.

The authors thank the Akwesasne Mohawk community for their collaboration and participation in the study of women's reproductive health. We are especially grateful to Tewentahawih 'tha' Cole, Beverly Cook, and Katsi Cook for their reading, review and comments on the manuscript. We also thank Craig Arquette on behalf of the advisory committee of the Akwesasne Task Force on the Environment for their review.

Relevant abbreviations and definitions

DDT	2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane
***p,p′*-DDE**	2,2-bis(*p*-chlorophenyl)-1,1-dichloroethylene
E_2	estradiol
FSH	Follicle stimulating hormone
HCB	Hexachlorobenzene
LH	Luteinizing hormone
OR	Odds ratio
P_4	Progesterone
PCBs	Polychlorinated biphenyls
POI	Primary ovarian insufficiency
POPs	Persistent organic pollutants

References

Abdalla H, Thum MY. An elevated basal FSH reflects a quantitative rather than qualitative decline of the ovarian reserve. Human Reproduction. 2004; 19:893–898. [PubMed: 15016786]

Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological Profile for Polychlorinated Biphenyls (PCBs). U.S. Department of Health and Human Services, Public Health Service; Atlanta, GA: 2000.

Agency for Toxic Substances and Disease Registry (ATSDR). Addendum to the Toxicological Profile for Polychlorinated Biphenyls (PCBs). US Department of Health and Human Services, Public Health Service; Atlanta, GA: 2011.

Aminov Z, Haase R, Rej R, Schymura MJ, Santiago-Rivera A, Morse G, DeCaprio A, et al. diabetes prevalence in relation to serum concentrations of polychlorinated biphenyl (PCB) congener groups and three chlorinated pesticides in a Native American population. Environ Health Perspect. 2016; 124:1376–1383. [PubMed: 27035469]

Ampleman MD, Martinez A, DeWall J, Rawn DFK, Hornbuckle KC, Thorne PS. Inhalation and dietary exposure to PCBs in urban and rural cohorts via congener-specific measurements. Environ Sci Technol. 2014; 49:1156–1164.

Axelrad DA, Goodman S, Woodruff TJ. PCB body burdens in us women of childbearing age 2001–2002: An evaluation of alternate summary metrics of nhanes data. Environmental Research. 2009; 109:368–378. [PubMed: 19251256]

Barroso G, Oehninger S, Monzo A, Kolm P, Gibbons WE, Muasher SJ. High FSH:LH ratio and low LH levels in basal cycle day 3: Impact on follicular development and IVF outcome. Journal of Assisted Reproduction and Genetics. 2001; 18:499–505. [PubMed: 11665665]

Bernert JT, Turner WE, Patterson DG Jr, Needham LL. Calculation of serum “total lipid” concentrations for the adjustment of persistent organohalogen toxicant measurements in human samples. Chemosphere. 2007; 68:824–831. [PubMed: 17408721]

Broekmans FJ, Kwee J, Hendriks DJ, Mol BW, Lambalk CB. A systematic review of tests predicting ovarian reserve and IVF outcome. Hum Reprod Update. 2006; 12:685–718. [PubMed: 16891297]

Broekmans FJ, Soules MR, Fauser BC. Ovarian aging: Mechanisms and clinical consequences. Endocrine Reviews. 2009; 30:465–493. [PubMed: 19589949]

Brown JF. Determination of PCB metabolic, excretion, and accumulation rates for use as indicators of biological response and relative risk. Environmental Science & Technology. 1994; 28:2295–2305. [PubMed: 22176047]

Buck GM, Sever LE, Batt RE, Mendola P. Life-style factors and female infertility. Epidemiology. 1997; 8:435–441. [PubMed: 9209860]

Buck GM, Sever LE, Mendola P, Zielezny M, Vena JE. Consumption of contaminated sport fish from Lake Ontario and time-to-pregnancy. New York State angler cohort. Am J Epidemiol. 1997; 146:949–954. [PubMed: 9400336]

Buck Louis GM, Lynch CD, Cooney MA. Environmental influences on female fecundity and fertility. Seminars in Reproductive Medicine. 2006; 24:147–155. [PubMed: 16804813]

Carpenter DO. 2015 Exposure to and health effects of volatile PCBs. Rev Environ Health. 30:81–92. [PubMed: 25822318]

Casey AC, Berger DF, Lombardo JP, Hunt A, Quimby F. Aroclor 1242 inhalation and ingestion by Sprague-Dawley rats. J Toxicol Environ Health, Part A. 1999; 56:311–342. [PubMed: 10094245]

Centers for Disease Control. Fourth National Report On Human Exposure To Environmental Chemicals. District of Columbia: Department of Health and Human Services; 2009.

Chambers GM, Sullivan EA, Ishihara O, Chapman MG, Adamson GD. The economic impact of assisted reproductive technology: A review of selected developed countries. Fertility and Sterility. 2009; 91:2281–2294. [PubMed: 19481642]

Chambers GM, Adamson GD, Eijkemans MJ. Acceptable cost for the patient and society. Fertility and Sterility. 2013; 100:319–327. [PubMed: 23905708]

Chandra A, Copen CE, Stephen EH. Infertility service use in the united states: Data from the national survey of family growth, 1982–2010. National Health Statistics Reports. 2014:1–21.

Connolly MP, Hoorens S, Chambers GM. Reproduction E, Society Task F. The costs and consequences of assisted reproductive technology: An economic perspective. Hum Reprod Update. 2010; 16:603–613. [PubMed: 20530804]

Cooke, PS., Sato, T., Buchanan, DL. Disruption of steroid hormone signaling by pcbs. In: Robertson, LW., Hansen, LG., editors. Pcbs recent advances in environmental toxicology and health effects. Louisville: University Press of Kentucky; 2001. p. 257-263.

Craig ZR, Wang W, Flaws JA. Endocrine-disrupting chemicals in ovarian function: Effects on steroidogenesis, metabolism and nuclear receptor signaling. Reproduction. 2011; 142:633–646. [PubMed: 21862696]

Currado GM, Harrad S. Comparison of polychlorinated biphenyl concentrations in indoor and outdoor air and the potential significance of inhalation as a human exposure pathway. Environ Sci Technol. 1998; 32:3042–3047.

Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, Hauser R, Prins GS, Soto AM, et al. Endocrine-disrupting chemicals: An endocrine society scientific statement. Endocrine Reviews. 2009; 30:293–342. [PubMed: 19502515]

Dumanoglu Y, Gaga EO, Gungormus E, Sofuoglu SC, Odabasi M. Spatial and seasonal variations, sources, air-soil exchange, and carcinogenic risk assessment for PAHs and PCBs in air and soil of Kutahya, Turkey, the province of thermal power plants. Sci Total Environ. 2017; 580:920–935. [PubMed: 27939936]

Forti, A., Bogdan, KG., Horn, E. Health risk assessment for the akwesasne mohawk population from exposure to chemical contaminants in fish and wildlife. Albany, NY: New York State Department of Health; 1995.

Foster WG, Neal MS, Han MS, Dominguez MM. Environmental contaminants and human infertility: Hypothesis or cause for concern? Journal Of Toxicology And Environmental Health Part B, Critical Reviews. 2008; 11:162–176.

Frederiksen M, Meyer HW, Ebbehoj NE, Gunnarsen L. Polychlorinated biphenyls (PCBs) in indoor air originating from sealants in contaminated and uncontaminated apartments within the same housing estate. Chemosphere. 2012; 89:473–479. [PubMed: 22763332]

Gallo MV, Ravenscroft J, Carpenter DO, Frye C, Cook B, et al. Akwesasne Task Force On The E. Endocrine disrupting chemicals and ovulation: Is there a relationship? Environmental Research. 2016; 151:410–418. [PubMed: 27543788]

Gillum RF, Sempos CT. Ethnic variation in validity of classification of overweight and obesity using self-reported weight and height in american women and men: The third national health and nutrition examination survey. Nutrition Journal. 2005; 4:27. [PubMed: 16209706]

Gougeon A, Ecochard R, Thalabard JC. Age-related changes of the population of human ovarian follicles: Increase in the disappearance rate of non-growing and early-growing follicles in aging women. Biology of Reproduction. 1994; 50:653–663. [PubMed: 8167237]

Gregoraszczuk EL, Ptak A, Karniewska M, Ropstad E. Action of defined mixtures of PCBs, p,p′-DDT and its metabolite p,p′-DDE, on co-culture of porcine theca and granulosa cells: steroid secretion, cell proliferation and apoptosis. Reprod Toxicol. 2008 Oct; 26(2):170–174. Epub 2008 Jul 22. DOI: 10.1016/j.reprotox.2008.07.003 [PubMed: 18692563]

Hansen, LG. The Ortho Side Of PCBs: Occurrence And Disposition. Norwell, MA: Kluwer Academic Publishers; 1999.

Hermanson MH, Johnson GW. Polychlorinated biphenyls in tree bark near a former manufacturing plant in Anniston, Alabama. Chemosphere. 2007; 68:191–198. [PubMed: 17307226]

Hernández-Ochoa I, Karman BN, Flaws JA. The role of the aryl hydrocarbon receptor in the female reproductive system. Biochemical Pharmacology. 2009 Feb 15; 77(4):547–59. [PubMed: 18977336]

Herrick RF, Meeker JD, Altshul L. Serum PCB levels and congener profiles among teachers in PCB-containing schools: a pilot study. Environ Health. 2011; 10:56. [PubMed: 21668970]

Hu X, Adamcakova-Dodd A, Lehmler HJ, Hu D, Hornbuckle K, Thorne PS. Subchronic inhalation exposure study of an airborne polychlorinated biphenyl mixture resembling the Chicago ambient air congener profile. Environmental Science & Technology. 2012; 46:9653–9662. [PubMed: 22846166]

Hu X, Adamcakova-Dodd A, Lehmler HJ, Hu D, Kania-Koirwel I, Hornbuckle KC, Thorne PS. Time course of congener uptake and elimination in rats after short-term inhalation exposure to an

airborne polychlorinated biphenyl (PCB) mixture. Environ Sci Technol. 2010; 44:6893–6900. [PubMed: 20698547]

Karwacka A, Zamkowska D, Radwan M, Jurewicz J. Exposure to modern, widespread environmental endocrine disrupting chemicals and their effect on the reproductive potential of women: An overview of current epidemiological evidence. Human Fertility (Camb). 2017; :1–24. Zjuly 31. Epub ahead of print. DOI: 10.1080/14647273.2017.1358828

Kouznetsova M, Huang X, Ma J, Lessner L, Carpenter DO. Increased rate of hospitalization for diabetes and residential proximity of hazardous waste sites. Environmental Health Perspectives. 2007; 115:75–79. [PubMed: 17366823]

Kronenberg, H., Williams, RH. Williams Textbook of Endocrinology. 11. Philadelphia: Saunders/Elsevier; 2008.

Kwintkiewicz J, Giudice LC. The interplay of insulin-like growth factors, gonadotropins, and endocrine disruptors in ovarian follicular development and function. Seminars In Reproductive Medicine. 2009; 27:43–51. [PubMed: 19197804]

Kwintkiewicz J, Nishi Y, Yanase T, Giudice LC. Peroxisome proliferator-activated receptor-gamma mediates bisphenol a inhibition of fsh-stimulated igf-1, aromatase, and estradiol in human granulosa cells. Environmental Health Perspectives. 2010; 118:400–406. [PubMed: 20064783]

Lacetti, G. General Motors/Central Foundry Division. New York: New York State Department of Health; 1993. Public health assessment.

Laden F, Neas LM, Spiegelman D, Hankinson SE, Willett WC, Ireland K, et al. Predictors of plasma concentrations of dde and pcbs in a group of U.S. women. Environmental Health Perspectives. 1999; 107:75–81. [PubMed: 9872720]

Lehmann GM, Christensen K, Maddaloni M, Phillips LJ. Evaluating health risks from inhaled polychlorinated biphenyls: Research needs for addressing uncertainty. Environmental Health Perspectives. 2015; 123:109–113. [PubMed: 25302536]

Liu KE, Greenblatt EM. Elevated day 3 follicle-stimulating hormone/luteinizing hormone ratio > 2 is associated with higher rates of cancellation in in vitro fertilization-embryo transfer cycles. Fertility and Sterility. 2008; 90:297–301. [PubMed: 18023434]

Lyu SW, Kim JW, Choi CH, Seok HH, Yoon TK, Kim A. Impact of high basal FSH/LH ratio in women with normal FSH levels on in vitro fertilization outcomes. Gynecological Endocrinology. 2013 May 1; 29(5):424–9. [PubMed: 23544714]

McFarland VA, Clarke JU. Environmental occurrence, abundance, and potential toxicity of polychlorinated biphenyl congeners: Considerations for a congener- specific analysis. Environmental Health Perspectives. 1989; 81:225–239. [PubMed: 2503374]

Melymuk L, Bohlin-Nizzetto P, Vojta S, Kratka M, Kukucka P, Audy O, et al. Distribution of legacy and emerging semivolatile organic compounds in five indoor matrices in a residential environment. Chemosphere. 2016; 153:179–186. [PubMed: 27016813]

Mendola P, Buck GM, Vena JE, Zielezny M, Sever LE. Consumption of PCB-contaminated sport fish and risk of spontaneous fetal death. Environmental Health Perspectives. 1995; 103:498–502. [PubMed: 7656880]

Mendola P, Buck GM, Sever LE, Zielezny M, Vena JE. Consumption of PCB-contaminated freshwater fish and shortened menstrual cycle length. Am J Epidemiol. 1997; 146:955–960. [PubMed: 9400337]

Minegishi T, Hirakawa T, Abe K, Kishi H, Miyamoto K. Effect of IGF-1 and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the expression of LH receptors during cell differentiation in cultured granulosa cells. Molecular And Cellular Endocrinology. 2003; 202:123–131. [PubMed: 12770741]

Nikolaou D, Templeton A. Early ovarian ageing: A hypothesis. Detection and clinical relevance. Hum Reprod. 2003; 18:1137–9. [PubMed: 12773436]

Patel S, Zhou C, Rattan S, Flaws JA. Effects of endocrine-disrupting chemicals on the ovary. Biology of Reproduction. 2015; 93:20. [PubMed: 26063868]

Persoon C, Peters TM, Kumar N, Hornbuckle KC. Spatial distribution of airborne polychlorinated biphenyls in Cleveland, Ohio and Chicago, Illinois. Environmental Science and Technology. 2010; 44:2797–2802. [PubMed: 20384374]

Phillips DL, Pirkle JL, Burse VW, Bernert JT Jr, Henderson LO, Needham LL. Chlorinated hydrocarbon levels in human serum: Effects of fasting and feeding. Archives Of Environmental Contamination And Toxicology. 1989; 18:495–500. [PubMed: 2505694]

Pliskova M, Vondracek J, Canton RF, Nera J, Kocan A, Petrik J, et al. Impact of polychlorinated biphenyls contamination on estrogenic activity in human male serum. Environmental Health Perspectives. 2005; 113:1277–1284. [PubMed: 16203234]

Pocar P, Brevini TA, Fischer B, Gandolfi F. The impact of endocrine disruptors on oocyte competence. Reproduction. 2003; 125:313–325. [PubMed: 12611595]

Pocar P, Fiandanese N, Secchi C, Berrini A, Fischer B, Schmidt JS, et al. Effects of polychlorinated biphenyls in CD-1 mice: Reproductive Toxicity And Intergenerational Transmission. Toxicological Sciences: An Official Journal of The Society Of Toxicology. 2012; 126:213–226. [PubMed: 22157103]

Pollack AZ, Buck Louis GM, Lynch CD, Kostyniak PJ. Persistent organochlorine exposure and pregnancy loss: A prospective cohort study. Journal Of Environmental Protection. 2011; 2:683–691. [PubMed: 22140635]

Prasad S, Gupta T, Divya A. Correlation of the day 3 FSH/LH ratio and LH concentration in predicting IVF outcome. Journal of Reproduction & Infertility. 2013; 14:23–28. [PubMed: 23926557]

Robertson LW, Ledewig G. Polychlorinated biphenyl (PCB) carcinogenicity with special emphasis on airborne PCBs. Gefahst Reinhalt Luft. 2011; 71:25–32.

Safe SH. Polychlorinated biphenyls (pcbs) and polybrominated biphenyls (pbbs): Biochemistry, toxicology, and mechanism of action. Critical Reviews In Toxicology. 1984; 13:319–395. [PubMed: 6091997]

Safe SH. Environmental and dietary estrogens and human health: Is there a problem? Environmental Health Perspectives. 1995; 103:346–351. [PubMed: 7607134]

Safe, SH. PCBs as Aryl hydrocarbon receptor agonists. In: Robertson, LW., Hansen, LG., editors. PCBs Recent Advances In Environmental Toxicology And Health Effects. Louisville: University Press of Kentucky; 2001. p. 171-177.

Safe SH. Endocrine disruptors and human health: Is there a problem. Toxicology. 2004; 205:3–10. [PubMed: 15458784]

Sallmen M, Weinberg CR, Baird DD, Lindbohm ML, Wilcox AJ. Has human fertility declined over time?: Why we may never know. Epidemiology. 2005; 16:494–499. [PubMed: 15951667]

Sandy AL, Guo J, Miskewitz RJ, McGillis WR, Rodenburg AL. Fluxes of polychlorinated biphenyls volatilizing from the Hudson River, New York, measured using micrometeorological approaches. Environ Sci Technol. 2012; 46:885–891. [PubMed: 22191600]

Schisterman EF, Whitcomb BW, Louis GM, Louis TA. Lipid adjustment in the analysis of environmental contaminants and human health risks. Environmental Health Perspectives. 2005; 113:853–857. [PubMed: 16002372]

Seckin B, Turkcapar F, Ozaksit G. Elevated day 3 FSH/LH ratio: A marker to predict IVF outcome in young and older women. Journal of Assisted Reproduction and Genetics. 2012; 29:231–236. [PubMed: 22183503]

Shi Z, Valdez KE, Ting AY, Franczak A, Gum SL, Petroff BK. Ovarian endocrine disruption underlies premature reproductive senescence following environmentally relevant chronic exposure to the Aryl hydrocarbon receptor agonist 2,3,7,8-tetrachlorodibenzo-p-dioxin. Biology of Reproduction. 2007; 76:198–202. [PubMed: 17050859]

Shirota M, Mukai M, Sakurada Y, Doyama A, Inoue K, Haishima A, et al. Effects of vertically transferred 3,3′,4,4′,5-pentachlorobiphenyl (PCB-126) on the reproductive development of female rats. J Reprod Dev. 2006; 52:751–761. [PubMed: 16983182]

Shrim A, Elizur SE, Seidman DS, Rabinovici J, Wiser A, Dor J. Elevated day 3 FSH/LH ratio due to low LH concentrations predicts reduced ovarian response. Reproductive Biomedicine online. 2006 Jan 1; 12(4):418–22. http://www.rbmojournal.com/article/S1472-6483(10)61993-1/pdf. [PubMed: 16740213]

Sifakis S, Androutsopoulos VP, Tsatsakis AM, Spandidos DA. Human exposure to endocrine disrupting chemicals: Effects on the male and female reproductive systems. Environmental Toxicology And Pharmacology. 2017; 51:56–70. [PubMed: 28292651]

Son WY, Das M, Shalom-Paz E, Holzer H. Mechanisms of follicle selection and development. Minerva Ginecologica. 2011; 63:89–102. [PubMed: 21508900]

Sunderam S, Kissin DM, Crawford SB, Folger SG, Jamieson DJ, Warner L, et al. Assisted reproductive technology surveillance - United States, 2013. Morbidity and Mortality Weekly Report Surveillance Summaries. 2015; 64:1–25.

Tang W, Aggarwal A, Moudon AV, Drewnowski A. Self-reported and measured weights and heights among adults in seattle and king county. BMC Obes. 2016; 3:11. [PubMed: 26918195]

Tsuchiya Y, Nakajima M, Yokoi T. Cytochrome P450-mediated metabolism of estrogens and its regulation in human. Cancer Lett. 2005; 227:115–124. [PubMed: 16112414]

Toft G, Hagmar L, Giwercman A, Bonde JP. Epidemiological evidence on reproductive effects of persistent organochlorines in humans. Reproductive Toxicology. 2004; 19:5–26. [PubMed: 15336708]

Toft G. Persistent organochlorine pollutants and human reproductive health. Danish Medical Journal. 2014; 61:B4967. [PubMed: 25370968]

Vabre P, Gatimel N, Moreau J, Gayrard V, Picard-Hagen N, Parinaud J, et al. Environmental pollutants, a possible etiology for premature ovarian insufficiency: A narrative review of animal and human data. Environmental health: A Global Access Science Source. 2017; 16:37. [PubMed: 28388912]

Windham GC, Elkin E, Fenster L, Waller K, Anderson M, Mitchell PR, et al. Ovarian hormones in premenopausal women: Variation by demographic, reproductive and menstrual cycle characteristics. Epidemiology. 2002; 13:675–684. [PubMed: 12410009]

Wolff MS, Camann D, Gammon M, Stellman SD. Proposed PCB congener groupings for epidemiological studies. Environmental Health Perspectives. 1997; 105:13–14. [PubMed: 9074863]

Yonker JA, Chang V, Roetker NS, Hauser TS, Hauser RM, Atwood CS. Hypothalamic–pituitary–gonadal axis homeostasis predicts longevity. Age. 2013 Feb 1; 35(1):129–38. [PubMed: 22139381]

Yu ML, Guo YL, Hsu CC, Rogan WJ. Menstruation and reproduction in women with polychlorinated biphenyl (PCB) poisoning: Long-term follow-up interviews of the women from the Taiwan Yucheng cohort. International Journal of Epidemiology. 2000; 29:672–677. [PubMed: 10922344]

Highlights

- Endocrine disrupting chemicals pose a threat to female fecundity and reproduction.
- Examined organochlorines and a marker of ovarian responsivity, FSH:LH ratio.
- Increasing concentrations of estrogenic PCBs predict FSH:LH ratio > 2.
- PCB congeners related to airborne exposure are associated with FSH:LH ratio > 2.

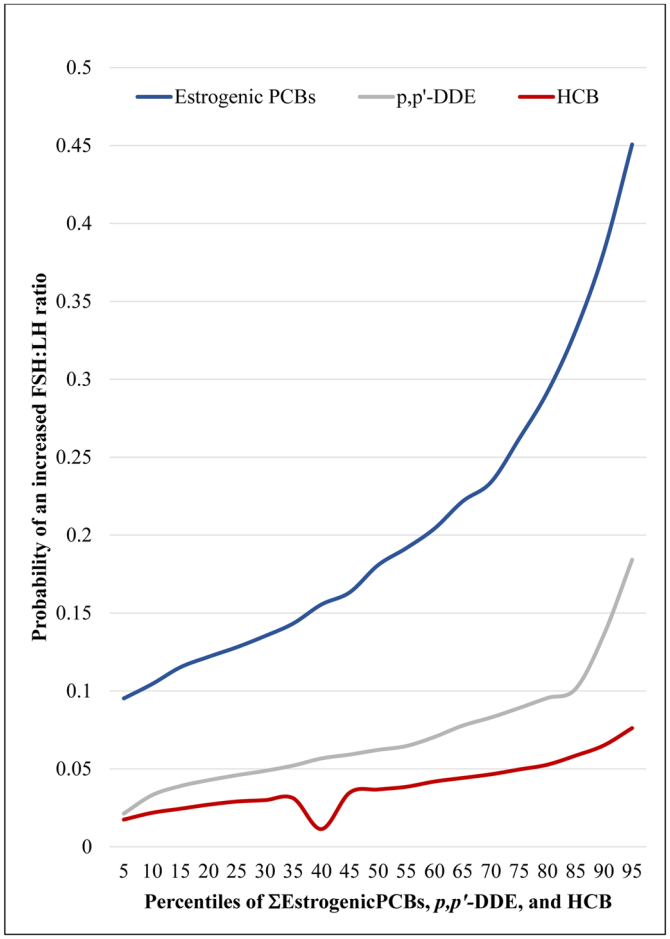

Figure 1.

Probability of an increased FSH:LH ratio in relation to estrogenic PCBs[a], *p,p′*-DDE and HCB in Akwesasne women (n=169).

[a]Sum of estrogenic PCBs (ppb; mdl/√2) CBs: 28, 31, 44, 47[59], 49, 52, 66, 70, 74, 95, 99, 101, 105, 110, 136, 153, 174, 177, and 187.

Table 1

Characteristics of Akwesasne Mohawk women (n=169)

	Mean	Median	SD	Max	% yes
Age (years)	30.3	30.3	4.89	39.2	
BMI (kg/m^2)	31.8	31.6	7.19	55.9	
% Body fat	39.2	40.5	4.83	47.76	
Education (yrs)	14.0	14.0	1.67	17.0	
Parity	1.3	1.0	1.40	6.0	
Cholesterol (mg/dL)	173.6	169.0	32.80	257.0	
Triglycerides (mg/dL)	131.3	102.0	136.17	1365.0	
Total lipids (mg/dL)	526.0	491.6	178.07	1939.9	
Marital Status *					48.0
Smoke cigarettes (in the past year)					44.4
Consumed alcohol (in the past year)					88.6
Overweight **					23.2
Obese ***					57.1

* Married or living with a partner

** Overweight defined as having a BMI between 25.0 – 29.9 kg/m^2

*** Obese defined as having a BMI ≥ 30.0 kg/m^2

Table 2

Cycle day 3 hormone levels among Akwesasne Mohawk women (n=169)

	All women					FSH:LH <2			FSH:LH ≥ 2			
	Mean	**SD**	**Median**	**Min**	**Max**	**Mean**	**SD**	**Median**	**Mean**	**SD**	**Median**	***p*-value***
Estradiol E2 (pg/mL)	40.6	23.32	36.1	7.2	207.2	41.9	24.19	37.5	31.6	13.06	30.2	0.06
Progesterone P4 (ng/mL)	0.8	1.27	0.6	0.1	14.4	0.9	1.35	0.7	0.6	0.28	0.6	0.45
Follicle stimulating hormone FSH (mIU/mL)	6.4	1.98	6.1	1.8	16.6	6.3	1.61	6.1	7.7	3.46	6.2	0.09
Luteinizing hormone LH (mIU/mL)	5.5	2.49	5.0	1.3	17.9	5.9	2.41	5.4	3.0	1.28	2.7	**≤0.01**

* *p*-value denotes the significant differences between women in the FSH:LH <2 and FSH:LH ≥ 2 groups

Table 3

PCB levels in all participants, and in women with FSH:LH ratio <2 or ≥ 2 (n=169).

	All women					FSH:LH <2				FSH:LH ≥ 2				
PCB grouping (ppb)	**GM**	**SD**	**Median**	**Min**	**Max**	**GM**	**SD**	**Median**	**Max**	**GM**	**SD**	**Median**	**Max**	***p*-value***
ΣMonoOrthoPCBs[a]	0.16	0.138	0.17	0.05	1.19	0.16	0.112	0.16	0.97	0.23	0.242	0.22	1.19	**≤0.01**
ΣDiOrthoPCBs[b]	0.57	0.326	0.52	0.18	1.98	0.56	0.299	0.52	1.95	0.64	0.467	0.66	1.98	0.18
ΣTriTetraOrthoPCBs[c]	0.16	0.199	0.13	0.05	1.08	0.15	0.185	0.13	1.08	0.21	0.272	0.16	0.97	**0.04**
ΣAirBornePCBs[d]	0.08	0.074	0.07	0.01	0.64	0.07	0.056	0.07	0.30	0.10	0.140	0.11	0.64	**0.02**
ΣEstrogenicPCBs[e]	0.39	0.304	0.38	0.13	2.11	0.38	0.260	0.35	2.10	0.52	0.494	0.47	2.11	**≤0.01**
Organochlorine pesticides (ppb)														
p,p′-DDE	0.30	0.334	0.29	0.06	3.50	0.29	0.344	0.29	3.50	0.31	0.266	0.30	1.23	0.65
HCB	0.02	0.013	0.02	0.003	0.1	0.02	0.013	0.02	0.10	0.02	0.012	0.03	0.05	0.33

[a] ΣMonoOrthoPCBs CBs: 28, 31, 33, 56, 63, 66, 70, 74, 105, 114, 118, and 156.

[b] ΣDiOrthoPCBs CBs: 32[16], 52, 49, 47+59, 44, 42, 71, 64, 92, 90+101, 99, 83, 87, 110, 138[164+163], 153, 158, 128, 172, 180, 170, and 190.

[c] ΣTriTetraOrthoPCBs CBs: 53, 51, 95, 91, 84, 151, 144, 187, 183, 185, 174, 177, 199, 203, and 136.

[d] ΣAirBornePCBs CBs: 28, 52, 66, and 74.

[e] ΣEstrogenicPCBs CBs: 28, 31, 44, 47, 49, 52, 66, 70, 74, 95, 99, 101, 105, 110, 136, 153, 174, 177, and 187.

* *p*-value denotes significance difference between women with FSH:LH ratio <2 or ≥ 2

Table 4

Results of the relationship between PCB groups and FSH:LH ratio from logistic regression model

Groupings		β	Wald	*p*-value	Exp(B)	95% CI for Exp (B)		Nagelkerke R Square	Chi-square
						Lower	Upper		
ΣMonoOrthoPCBs[a]		1.61	9.39	***≤0.01***	5.01	1.79	14.03	0.33	16.38
ΣDiOrthoPCBs[b]		1.14	3.58	0.06	3.13	0.96	10.18	0.25	16.96
ΣTriTetraOrthoPCBs[c]		0.82	4.39	***0.04***	2.28	1.06	4.92	0.27	6.14
ΣAirBornePCBs[d]		0.98	5.10	***0.02***	2.66	1.14	6.19	0.28	5.93
ΣEstrogenicPCBs[e]		1.58	8.81	***≤0.01***	4.85	1.71	13.76	0.31	10.59
PCB congeners	ROD								
47[59][f]	31.52%	1.30	11.28	***≤0.01***	3.67	1.72	7.85	0.34	9.95
52	88.04%	0.74	4.02	***0.05***	2.10	1.02	4.34	0.27	5.02
95	35.87%	0.41	3.76	***0.05***	1.51	1.00	2.28	0.26	12.62
101[90][f]	92.93%	0.82	6.07	***0.01***	2.28	1.18	4.38	0.29	16.74
105	63.04%	0.62	8.49	***≤0.01***	1.86	1.23	2.84	0.32	9.91

ROD: Rate of detection

[a] ΣMonoOrthoPCBs CBs: 28, 31, 33, 56, 63, 66, 70, 74, 105, 114, 118, and 156.

[b] ΣDiOrthoPCBs CBs: 32[16], 52, 49, 47+59, 44, 42, 71, 64, 92, 90+101, 99, 83, 87, 110, 138[164+163], 153, 158, 128, 172, 180, 170, and 190.

[c] ΣTriTetraOrthoPCBs CBs: 53, 51, 95, 91, 84, 151, 144, 187, 183, 185, 174, 177, 199, 203, and 136.

[d] ΣAirBornePCBs CBs: 28, 52, 66, and 74.

[e] ΣEstrogenicPCBs CBs: 28, 31, 44, 47, 49, 52, 66, 70, 74, 95, 99, 101, 105, 110, 136, 153, 174, 177, and 187.

[f] Bracket indicates 'minor' congener based on Aroclor concentration (Hansen, 1999).