Hormone Disruption, Bone Mineral Density, and Persistent Organic Pollutants in Postmenopausal Women

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THESIS

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This work is dedicated to my husband and son, Jaime and Nicholas Pimentel.

This work is also dedicated to the charter boat captains, anglers, and United States residents who participated in the research projects on which these studies are based.

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LIST OF ABBREVIATIONS

AhR	Aryl hydrocarbon receptor
BMD	Bone mineral density
BMI	Body mass index
CDC	Centers for Disease Control and Prevention
CRP	C-reactive protein
CV	Coefficients of variation
CYP19	Cytochrome P450
DXA	Dual-energy X-ray absorptiometry
E2	Estradiol
FM	Fat mass
FSH	Follicle-stimulating hormone
FSH:E2	The ratio of follicle-stimulating hormone to estradiol
GGT	Gamma-glutamyl transferase
GLFCS	Great Lakes Fish Consumption Study
HPG	Hypothalamo-pituitary-gonadal
LH	Luteinizing hormone
Ln	Natural log
LOD	Limit of detection
NHANES	National Health and Nutrition Examination Survey
PCBs	Polychlorinated biphenyls
PCDDs	Polychlorinated dibenzo-p-dioxins
PCDFs	Polychlorinated dibenzofurans
PIR	Poverty-to-income ratio
POPs	Persistent organic pollutants
SERMs	Selective estrogen receptor modulators
SES	Socioeconomic status
SHBG	Sex hormone-binding globulin
SHBG-E2	Sex hormone-binding globulin-bound estradiol
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin

TEQs	Toxic equivalents
TZDs	Thiazolidinediones

SUMMARY

The goals of this research were to examine the associations of persistent organic pollutants (POPs), including polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs), with both endogenous hormones and bone mineral density (BMD) among postmenopausal women not taking glucocorticoids or sex hormones. Our investigations focused on the relationships of BMD and hormones with POP congeners grouped into categories with similar structure or mechanism of action using two sources of data with differing demographics and POP body burdens: the National Health and Nutrition Examination Survey (NHANES) and the Great Lakes Fish Consumption Study (GLFCS). We hypothesized that associations of POP body burdens with endpoints would be modified by factors that may affect endogenous hormone profile.

Our findings were somewhat consistent with an increasing body of literature suggesting an estrogenic effect of POP body burdens. We found some but not consistent evidence of a dose-response increase in BMD with exposure to both estrogenic and/or non-dioxin-like POPs and anti-estrogenic and/or dioxin-like POPs among 603 NHANES participants. We also found inverse associations of luteinizing hormone (LH), but not follicle-stimulating hormone (FSH), with exposure to anti-estrogenic and/or dioxin-like POPs among 89 participants in the NHANES. Gamma-glutamyl transferase (GGT), a potential confounder, attenuated these associations. Finally, we found inverse associations of FSH and sex hormone-binding globulin (SHBG) with exposure to estrogenic and/or non-dioxin-like PCBs among 77 women participating in the GLFCS.

Associations were enhanced by potential effect modifiers related to both direct and indirect estrogenicity, including hypothyroidism, obesity, and the obesity-related conditions diabetes and inflammation identified by elevated C-reactive protein (CRP). The metabolism of both androgens and estrogens is affected in hypothyroidism. Androgen production is decreased, and the metabolism of testosterone is shifted toward etiocholanolone rather than androsterone. With respect to estradiol and estrone, hypothyroidism favors metabolism of these steroids via the more estrogenic 16α-hydroxylation rather than 2-hydroxylation. Sex hormone-binding globulin in circulation is also decreased, resulting in decreased levels of estradiol, but the bioavailable fraction is increased. Because aromatization of androgen precursors to estrogen occurs predominately in peripheral fat, obese women have higher estrogen levels and are therefore at lower risk of diseases related to estrogen deficiency, such as osteoporosis. Obesity is also associated with decreased SHBG resulting in an increased fraction of bioavailable estrogen. Obesity has been related to elevated CRP and is also a major risk factor influencing the risk of type 2 diabetes.

Some of our findings were inconsistent with previous investigations, suggesting an estrogenic effect of POP body burdens. We observed decreased LH with exposure to anti-estrogenic and/or dioxin-like POPs in the NHANES. Possible mechanisms to elucidate this finding include cross-talk between the aryl hydrocarbon receptor (AhR) and estrogen receptors and a prior investigation showing up-regulation of genes that code for aromatase and estrogen receptor beta with exposure to anti-estrogenic and dioxin-like PCBs. We noted positive associations of PCBs with estradiol, but associations were generally nonsignificant. Mechanisms by which POPs could affect estrogenicity exclusive of direct effects on estradiol may potentially relate to up-regulation of genes coding for estrogen receptors, alpha and beta, and/or direct effects on hormone receptors. Environmental chemicals acting as steroid hormones due to their similarity in structure may bind to estrogen receptors and stimulate transcription, and thus, production of proteins that mediate the effects of estrogen. We did not observe effect modification for the associations between PCBs and FSH, SHBG, and SHBG-bound estradiol (SHBG-E2). Estrone is the predominant form of estrogen in postmenopausal women, potentially explaining the lack of effect modification by estradiol.

Future epidemiologic investigations should evaluate effect modification by estrone, the main form of estrogen in postmenopausal women, as well as the major metabolites of estradiol and estrone, estriol, and catechol estrogens. Measures of centrally distributed body fat, such as waist-to-hip ratio, should also be evaluated in addition to other measures of adiposity.

I. PROJECT SUMMARY AND SPECIFIC AIMS

The overreaching theme of these investigations was to examine the relationships of common classification schemes of PCB, PCDD, and PCDF congeners with BMD and hormones among postmenopausal women not taking glucocorticoids or sex hormones. We hypothesized that associations of exposures with endpoints would be modified by factors affecting the estrogen profile of participants.

The analyses were conducted using data from two sources: (1) NHANES databases with cross-sectional health, exposure, and biomarker data; and (2) GLFCS, a cross-sectional follow-up study composed of frequent and infrequent sport fish consumers.

The specific aims of this thesis were:

1. To examine the associations between POPs and BMD in postmenopausal women.
2. To examine the associations of POPs with FSH and LH in postmenopausal women.
3. To examine the associations between PCBs and FSH, estradiol, SHBG, and SHBG-E2 in postmenopausal women.

II. BACKGROUND

A. Endocrine Disruption

Persistent organic pollutants, including PCBs, PCDDs, and PCDFs, are a complex group of environmental contaminants with potential endocrine-disrupting effects. An endocrine disruptor is defined as an exogenous agent that can interfere with various aspects of hormone synthesis, secretion, binding, transport, regulation, action, and elimination (Zoeller et al., 2012). The synthesis and secretion of steroid hormones are controlled by the positive and negative feedback mechanisms of the hypothalamo-pituitary-gonadal (HPG) axis, but it has been suggested that exposure to POPs may also result in modifications of circulating steroid hormone levels and, in turn, estrogen-dependent bone parameters.

B. Persistent Organic Pollutants

Among a class of heat-resistant, oily liquids, PCBs were used as insulating fluids in capacitors and transformers (ATSDR, 2000). Dioxins such as PCDDs and PCDFs are compounds formed as unintentional by-products of industrial processes (Fiedler, 1996). Like PCBs, dioxins are resistant to abiotic and biotic degradation in the environment and bioaccumulate and magnify in animals and humans (Safe, 1994; Van den Berg et al., 1998). Human body burdens of these chemicals have declined over time (Aylward and Hays, 2002; Turyk et al., 2012); however, continued exposure is principally through consumption of animal-derived fats (van Larebeke et al., 2001). Persistent organic pollutants in fatty, long-lived, predatory fish can accumulate and pass from one species to the next through the food chain. Frequent consumers of Great Lakes fish are at increased risk of exposure to these contaminants (Imm et al., 2005). In the GLFCS, duration and quantity of Great Lakes fish consumption was reflected in higher body burdens of PCBs (Hanrahan et al., 1999). Influenced by both the decline in PCB levels in fish and the decline in Great Lakes sport fish consumption, body burdens of contaminants in this cohort decreased over time (Knobeloch et al., 2009). Nonetheless, "PCB levels remained higher in Great Lakes sport-caught fish consumers in 2004–2005 than in a representative sample of the US population in 2003–2004" (Turyk et al., 2012).

Variations in action and toxicological properties of individual PCB, PCDD, and PCDF congeners have guided several investigators to construct groupings "with similar characteristics based on degree of chlorination, degree and type of enzyme induction, estrogenic and anti-estrogenic activities, prevalence in the environment, abundance in animal tissue and other toxicological characteristics" (Warner et al., 2012). These categories continue to evolve. Polychlorinated biphenyl, PCDD, and PCDF congeners have varying patterns of toxicity. The AhR has a high affinity for 2,3,7,8-substituted PCDDs and PCDFs in addition to some non- and mono-*ortho* substituted PCBs (Poland et al., 1985; Safe et al., 1985; Van den Berg et al., 1998). Biochemical and toxic responses mediated by the AhR are generally considered to be anti-estrogenic; however, dioxins have been shown to induce endometriosis and estrogen-dependent tumors (van Larebeke et al., 2001), suggesting cross-talk

between estrogen receptors and the AhR signaling pathways (Ohtake et al., 2003). In addition, dioxin-like, potentially anti-estrogenic PCBs were reported to up-regulate genes coding for the expression of receptors and enzymes involved in estrogen metabolism, including estrogen receptor beta and cytochrome P450 (CYP19), which encodes aromatase, the enzyme responsible for the conversion of androgen precursors to estrogen. Recently, it has been shown that dioxins and dioxin-like POPs up-regulate genes related to adiposity, insulin resistance, and inflammation (Kim et al., 2012). Non-dioxin-like POPs may act through other mechanisms not related to the AhR, while some PCBs may exhibit estrogenic effects by inhibiting estrogen sulfotransferase, the enzyme responsible for inhibiting estrogen metabolism (Kester et al., 2000; Kester et al., 2002).

C. Bone Mineral Density

Osteoporosis is a common affliction among older women that is characterized by low bone mass, microarchitectural disruption, and compromised bone strength resulting in an increased risk of fractures following minor trauma (Cummings and Melton III, 2002b). Osteoporosis occurs when the rate of bone resorption exceeds the rate of bone formation. The measurement of BMD by dual x-ray absorptiometry is the gold standard method to diagnose osteoporosis and is recommended for all postmenopausal women aged 65 years and older regardless of risk factors (Cummings et al., 2002a; Baim et al., 2008; National Osteoporosis Foundation, 2013).

Previous investigations have suggested androgens play an important role in bone physiology. Androgen deficiency leads to loss of bone density (Yasui et al., 2012). More importantly, bone is a target tissue for estrogen and estrogen deficiency appears to be an important factor in the osteoporotic process (D'Amelio et al., 2008). Further, estrogen level has been shown to influence the toxicity of POPs on bone in three experimental investigations. First, studies in rats exposed to PCB 126 showed that bone metabolism was affected differently depending on estrogen status. Exposure to PCB 126 did not affect BMD or trabecular bone volume of the tibia in sham-operated female rats. Conversely, in estrogen-deprived ovariectomized rats, exposure to PCB 126 resulted in increased BMD of the tibia or an indication of agonistic activity of the effect of PCB 126 on BMD (Lind et al., 1999). Second, in ovariectomized rats, PCB 126 together with estradiol supplementation increased trabecular bone volume, while the opposite was found in sham-operated rats (Lind et al., 2004). Finally, estrogenic and anti-estrogenic aroclors were found to differentially interfere with bone turnover mechanisms, particularly in ovariectomized rats (Yilmaz et al., 2006).

D. Hormones

Steroid and gonadotropin hormone function is regulated by the HPG axis. Any associations of POPs with steroid or gonadotropin hormones may be more clearly observed among postmenopausal women who are under less influence of ovarian

estrogen. Menopause is defined as one year after the permanent cessation of menses, which women experience at the average age of 51 years. With ovarian follicular depletion, the decline in estrogen production by the ovaries lessens the negative feedback on the hypothalamus and pituitary, resulting in increased FSH levels. Estrogen production in postmenopausal women does not stop completely because production at low levels continues throughout life. "The main source of estrogen after menopause is from the aromatization of androgenic precursors, a reaction that is catalyzed by the aromatase enzyme and predominantly occurs in the adipose tissue" (Mullin et al., 2011). Overweight women convert more androgens to estrogens when compared with women of normal weight because androgens are converted to estrogens in peripheral adipose tissue. Therefore, overweight women are thought to have a decreased risk for diseases associated with estrogen deficiency, such as osteoporosis. In circulation, estrogens are primarily bound to proteins known as SHBG, but also to albumin. Hepatic synthesis of SHBG is affected by the estrogen-androgen balance, with estrogens stimulating and androgens suppressing their production (Bulun and Adashi, 2008). Other health-related factors altering SHBG levels, such as hypothyroidism, obesity, and inflammation, may influence the bioavailability of estradiol.

A limited but growing number of epidemiologic investigations have been conducted to determine the effects of POP exposure on hormonal balance. Epidemiologic investigations have also explored possible associations of PCBs with steroid hormones in men, with only one known study in women. In 75 postmenopausal women occupationally exposed to PCBs at a capacitor manufacturing plant in LaSalle, Illinois, inverse associations were found for PCBs with FSH and SHBG (Persky et al., 2011). In a study of 178 men participating in the GLFCS, there were significant associations of PCBs with SHBG-bound testosterone, but not with SHBG or free testosterone. In addition, no associations with estrone sulfate, FSH, LH, or testosterone were found (Persky et al., 2001). In an analysis that examined "the effects of exposure to dioxin-like chemicals on thyroid and steroid hormones in a subgroup of 56 men from the [same] Great Lakes cohort," a negative relationship between SHBG-bound testosterone levels and total noncoplanar PCBs was observed, but no association was seen between SHBG and PCBs (Turyk et al., 2006). In a study of 305 young Swedish men, SHBG-bound testosterone was negatively associated with PCB 153 (Richthoff et al., 2003). In 197 men living in Norway, a significant positive association between PCB 153 and SHBG was found and remained after adjusting for age and body mass index (BMI), suggesting an effect on protein level (Haugen et al., 2011). In several recent studies of men, PCB 153 was significantly and positively associated with SHBG levels; however, this relationship was no longer evident after adjustment for age (Hagmar et al., 2001; Richthoff et al., 2003; Rignell-Hydbom et al., 2004). An analysis of steroid hormones within four populations indicated several endocrine responses associated with PCB 153 levels in some countries' populations (Greenland and Ukraine) but not in others (Poland and Greenland Inuit). A weak elevation in FSH with increasing PCB 153 was observed in each group but the pooled analysis was not statistically significant (Giwercman et al., 2006). Although the epidemiologic data have yielded inconsistent results, an increasing number of experimental studies give

biological plausibility to POP-induced hormone-disrupting effects. In male rats, acute exposure to PCB 153 resulted in decreased FSH and LH concentrations (Desaulniers et al., 1999). According to Hodgson et al. (2008), "The effect of exposure to a number of different organochlorines is likely to be difficult to predict; nonetheless, these animal studies suggest a possible causal relationship between organochlorine exposure and [hormone-disrupting] effects."

III. ASSOCIATIONS OF PERSISTENT ORGANIC POLLUTANTS AND BONE MINERAL DENSITY IN POSTMENOPAUSAL WOMEN: NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY 1999–2004

Persistent organic pollutants, such as PCBs, PCDDs, and PCDFs, are a class of environmental contaminants with the potential for endocrine-disrupting effects. Despite declining human body burdens over time (Aylward and Hays, 2002; Turyk et al., 2012), the US population continues to be exposed to low levels of POPs, primarily through the ingestion of contaminated food items (van Larebeke et al., 2001).

Persistent organic pollutants have been shown to exhibit a broad range of toxic effects including disruption of sex hormone homeostasis. Previous reports have demonstrated the estrogenic and anti-estrogenic properties of some POPs. Dioxin-like POPs, including PCDDs, PCDFs, and some mono- and non-*ortho* PCBs, exert their anti-estrogenic effects by binding to the AhR (Van den Berg et al., 1998), while non-dioxin-like POPs may act through other mechanisms. Dioxins have been shown to induce endometriosis and estrogen-related tumors (van Larebeke et al., 2001), suggesting cross-talk between the estrogen receptor and the AhR signaling pathways (Ohtake et al., 2003). In addition, PCBs were reported to up-regulate the expression of receptors and enzymes involved in steroid metabolism, including estrogen receptor beta and CYP19), which encodes aromatase, the enzyme that converts androgen precursors to estrogen (Warner et al., 2012). It has recently been suggested that dioxins and dioxin-like POPs up-regulate the expression of genes related to adiposity, insulin resistance, and inflammation (Kim et al., 2012). Whether any of these mechanisms are relevant for POP effects on BMD is presently unknown. Conversely, some PCBs bear a resemblance to estradiol in terms of chemical structure and, thus, a potential mechanism for estrogenic PCBs includes the capacity to bind and activate estrogen receptors (Cooke et al., 2001) or bind to serum binding proteins (Hodgert et al., 2000). In addition, some PCBs and their metabolites inhibit estrogen sulfotransferase, the enzyme known to inhibit estrogen metabolism (Kester et al., 2000; Kester et al., 2002).

There is evidence to show that androgens are important in bone physiology. Androgen deficiency appears to reduce bone density (Yasui et al., 2012).

> "It is well established from both human and experimental animal models that estrogen plays an important role for bone homeostasis. A marked decrease in bone mineral density is seen following ovariectomy in humans. Furthermore bone mineral loss in women is markedly increased in the years following menopause, when the circulatory levels of estrogen are reduced" (Lind et al., 1999).

Hormone replacement therapy with estrogens decreases the rate of further bone loss. Additionally, the protein binding of steroid hormones affects their bioactivity and clearance rates to different degrees. Estradiol unbound to

SHBG appears to be the best parameter of bioactive estradiol in describing its positive relationship with bone characteristics (van den Beld et al., 2000; Khosla et al., 2001; Van Pottelbergh et al., 2003; Lapauw et al., 2009).

There is a growing body of literature showing associations of POPs with bone parameters in humans, but results have not been consistent. One investigation of the relationship of POPs and adverse bone effects in humans focused on populations with high exposures (Miller, 1985). In several studies, POP body burdens were not associated with bone effects after adjusting for important confounders (Bohannon et al., 2000; Wallin et al., 2005; Cote et al., 2006), while another study suggested an inverse association between POPs and BMD (Glynn et al., 2000). Other investigations suggested an association between POP exposure and increased BMD (Hodgson et al., 2008; Rignell-Hydbom et al., 2009; Cho et al., 2011) with evidence that associations were positive among older women with high fat mass (FM) and inversely associated among older women with low FM (Cho et al., 2011). In a recent investigation, postnatal 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposure, one of the most toxic PCDD congeners, was associated with improved bone measures in postmenopausal women heavily exposed in Seveso, Italy (Eskenazi et al., 2013). A number of factors may be related to the inconsistent findings, such as varied detection methods for POPs and bone parameters, differing populations, dose, type of exposure evaluated, and chemical mixture, as well as differences in factors related to estrogen profile.

The purpose of our study was to expand on previous analyses of the 1999–2004 NHANES that investigated the association of POPs with BMD and whether associations of body composition with BMD were modified by POPs (Cho et al., 2011). The NHANES is a cross-sectional survey examining a random sample of the US population (CDC, 2014). The present study uses dual-energy X-ray absorptiometry (DXA) BMD, PCB, PCDD, and PCDF data obtained in the 1999–2000, 2001–2002, and 2003–2004 survey cycles. We examined the effects of general population-level POP exposures on BMD in a subgroup of postmenopausal women not taking glucocorticoids or sex hormones. “Any association between POPs and BMD may be more clearly observed among postmenopausal women who are under less influence of endogenous [or exogenous] estrogen” (Cho et al., 2011) and at higher risk of osteoporosis. This report is focused on the dose-response relationships of BMD with dioxin-like toxic equivalents (TEQs), several individual PCB congeners, ∑PCBs, and PCB congeners grouped into categories with structural similarity or mechanism of action. In addition, we examined the hypothesis that associations of POPs with BMD are modified by factors that may influence estrogen profile, such as age, BMI, and use of certain medications.

A. Methods

1. Participants

We obtained the 1999–2000, 2001–2002, and 2003–2004 study cycle data sets online (CDC, 2014). Each survey is a nationally representative sample of the US civilian, noninstitutionalized population based on a complex probability sampling design. Menopause is defined as one year after the permanent cessation of menses, which women experience at the average age of 51 years. Participants with the absence of menstrual periods for 12 months or more specified the reason for not having regular periods as "going/gone through menopause" (1999–2000 and 2001–2002 study cycles) or for not having periods as "menopause/hysterectomy" (2003–2004 study cycle). The following criterion was used to define menopause: any age and last period ≥12 months with or without hysterectomy or bilateral oophorectomy.

In the present investigation, we focused on the 953 postmenopausal participants ≥40 years of age with questionnaire data that included information on hysterectomy and bilateral oophorectomy. We excluded postmenopausal participants who were <40 years of age because they were assumed to have experienced early menopause. Women with premature menopause are at higher risk for osteoporosis and fractures (National Osteoporosis Foundation, 2013). We excluded postmenopausal participants <56 years of age who specified hysterectomy without oophorectomy (*n*=41). Participants were excluded if they did not have exposure and BMD measures (*n*=109). Complete data for analysis of the associations of POPs with BMD were available for a total of 603 participants after excluding participants missing data for cotinine level (*n*=5); BMI (*n*=3); or prescription medication use (*n*=1); or those who specified taking sex hormones (estrogen, progestins, sex hormone combinations, miscellaneous sex hormones, gonadotropin-releasing hormone and analogs, androgens and anabolic steroids, and contraceptives), other hormones/hormone modifiers (selective estrogen receptor modulators [SERMs], aromatase inhibitors, antiandrogens, and antigonadotropic agents) (*n*=215), or glucocorticoids (*n*=17) in the past 30 days. Both hormone replacement therapy and SERMs are effective in the treatment of osteoporosis, while glucocorticoids are potent osteopenic agents (Rosen and Drezner, 2013).

2. Bone mineral density and other physiological measurements

The DXA examination protocol has been previously described (CDC, 2004). Briefly, BMD was measured using whole-body DXA scans (Hologic QDR® 4500A fan-beam densitometer, Hologic, Inc., Bedford, Massachusetts. Scans for each subject were reviewed and analyzed by the University of California, San Francisco, Department of Radiology applying standard radiologic methods. Highly variable supplemental data were not included in this analysis. Osteoporosis is defined as "a chronic, progressive disease characterized by low bone mass, microarchitectural deterioration of bone tissue and decreased bone strength, bone fragility, and a consequent increase in fracture risk," particularly at the hip, spine, and wrist, although any bone can be affected (National Osteoporosis Foundation, 2013). As a result, we selected left arm, left leg, thoracic spine, lumbar

spine, and pelvis BMD sites for this report. Because the right side is the dominant side in the majority of individuals (Beaton, 2003), analyses were repeated with right-side extremities. These analyses yielded similar results (data not shown).

Details of the NHANES laboratory measurements are available online for 1999–2000 (CDC, 2010a), 2001–2002 (CDC, 2010b), and 2003–2004 (CDC, 2013). C-reactive protein was measured by latex-enhanced nephelometry with high sensitivity using a Dade Behring Nephelometer II Analyzer System (Dade Behring Diagnostics, Inc., Somerville, New Jersey). Serum total cholesterol was measured enzymatically after hydrolyzation and oxidation, while triglycerides were analyzed enzymatically after hydrolyzation into glycerol. Serum cotinine levels were measured by isotope dilution-high performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry. There was no limit of detection (LOD) reported.

3. Polychlorinated biphenyl, polychlorinated dibenzo-p-dioxin, and polychlorinated dibenzofuran measurements

Levels of POPs in serum were measured using high-resolution gas chromatography/isotope-dilution high-resolution mass spectrometry (Organic Toxicology Branch, National Center for Environmental Health, CDC, Atlanta, Georgia). The various congener groupings used in the analyses are listed in Table I. Toxic equivalents were calculated for non-*ortho* and mono-*ortho* PCB congeners, PCDDs, and PCDFs by summing "the products of the concentration of each compound multiplied by its [toxic equivalency factor] value" (Van den Berg et al., 2006) to yield ∑TEQs. Congener-specific values for PCB congeners were summed to yield ∑PCBs. We grouped PCB congeners according to structure including ∑non-dioxin-like PCBs, ∑mono-*ortho* PCBs, and ∑dioxin-like PCBs (consisting of non-*ortho* and mono-*ortho* PCBs). We also grouped PCB congeners according to mechanism of action, including estrogenic and anti-estrogenic activity (Wolff et al., 1997; Cooke et al., 2001). All of the congeners contributing to ∑Cooke anti-estrogenic PCBs were dioxin-like PCBs, while six out of eight congeners contributing to ∑Wolff anti-estrogenic PCBs were considered dioxin-like. In addition, dioxin-like PCB 126 is listed in both ∑Cooke estrogenic PCBs and ∑Cooke anti-estrogenic PCBs. Three PCB congeners, PCB 138, PCB 153, and PCB 180, were also selected for the analysis because they are most frequently detected in population studies (CDC, 2009). For congeners with results below the LOD, the measurement was imputed by the Centers for Disease Control and Prevention (CDC) as the LOD for each congener divided by the square root of two. The LOD differed for each participant since it was based on the volume of the sample. In the first study cycle (1999–2000), fewer congeners were assessed and more of the measurements were below the LOD compared with the second (2001–2002) and third (2003–2004) study cycles (Table II). Only congeners that had >10% of results >LOD for each of the three study cycles were included in the analysis. When measurements for more than one congener were not reported by the CDC for a participant, the participant was coded as missing for each summary exposure mentioned previously. However,

∑Wolff estrogenic PCBs comprised two congeners; therefore, the participant was coded as missing if one of the congeners was not reported by CDC.

TABLE I

CONGENER GROUPINGS, NHANES 1999–2004

Grouping	Congeners[a]
∑TEQs[b]	PCB congeners **105, 118, 126, 156, 167, 169**; PCDD congeners **1,2,3,7,8-PentaPCDD, 1,2,3,6,7,8-HexaPCDD, 1,2,3,7,8,9-HexaPCDD, 1,2,3,4,6,7,8-HeptaPCDD, 1,2,3,4,6,7,8,9-OctaPCDD**; PCDF congeners **2,3,4,7,8-PentaPCDF, 1,2,3,4,7,8-HexaPCDF, 1,2,3,6,7,8-HexaPCDF, 1,2,3,4,6,7,8-HeptaPCDF**
∑PCBs[b]	**66, 74, 99, 105, 118, 126, 138, 146, 153, 156, 167, 169, 170, 177, 178, 180, 183, 187**
∑Non-dioxin-like PCBs[b]	**66, 74, 99, 138, 146, 153, 170, 177, 178, 180, 183, 187**
∑Mono-*ortho* PCBs	**105**, 114, **118,** 123, **156**, 157, **167**, 189
∑Dioxin-like PCBs	77, 81, **105**, 114, **118**, 123, **126**, **156**, 157, **167**, **169**, 189
∑Cooke estrogenic PCBs	1, 3, 4, 8, 15, 18, 21, 31, 44, 47, 48, 49, 52, 54, 61, 70, 75,77, 80, 95, **99**, 101, 104, 110, **126**, 136, **153**, 155, 184, 188
∑Wolff estrogenic PCBs	101, 174, **177**, **187**, 201
∑Cooke anti-estrogenic PCBs	37, 77, 81, **105**, 114, **126**[c], 155, **156**, **169**
∑Wolff anti-estrogenic PCBs	**66**, **74**, 77, **105**, **118**, **126**[c], 155, **156**, **167**, **169**

[a]Congeners in bold were included in the grouping.
[b]Only measured congeners in the grouping are shown.
[c]PCB 126 is listed in both ∑Cooke estrogenic PCBs and ∑Cooke anti-estrogenic PCBs.

TABLE II

PERSISTENT ORGANIC POLLUTANTS FOR 603 ELIGIBLE POSTMENOPAUSAL WOMEN, NHANES 1999–2004

	Participants with results below detectable limit (percent)[a]		
POP[a]	**1999–2000 cycle**	**2001–2002 cycle**	**2003–2004 cycle**
PCB 66	87.9	85.5	1.6
PCB 74	4.0	3.3	0.0
PCB 99	24.2	11.4	0.0
PCB 105	53.2	51.3	0.0
PCB 118	7.0	4.9	0.0
PCB 126	19.6	10.0	0.2
PCB 138	14.1	0.4	0.0
PCB 146	35.7	23.3	0.0
PCB 153	9.0	0.9	0.0
PCB 156	24.8	10.9	0.0
PCB 167	84.1	75.0	1.6
PCB 169	11.8	3.5	2.6
PCB 170	10.6	1.2	0.0
PCB 177	89.1	63.8	0.0
PCB 178	82.1	66.5	0.0
PCB 180	6.9	1.5	0.0
PCB 183	64.8	47.1	0.0
PCB 187	11.9	8.0	0.0
1,2,3,7,8-PentaPCDD	78.5	49.2	15.9
1,2,3,6,7,8-HexaPCDD	41.0	1.2	5.6
1,2,3,7,8,9-HexaPCDD	63.4	48.0	45.3
1,2,3,4,6,7,8-HeptaPCDD	14.9	4.2	0.0
1,2,3,4,6,7,8,9-OctaPCDD	6.9	3.3	0.3
2,3,4,7,8-PentaPCDF	21.7	18.6	6.3
1,2,3,4,7,8-HexaPCDF	28.6	5.2	10.7
1,2,3,6,7,8-HexaPCDF	49.5	17.4	20.6
1,2,3,4,6,7,8-HeptaPCDF	45.0	10.8	9.6
∑TEQs[b]	0.0	0.0	0.0
∑PCBs[b]	0.0	0.0	0.0
∑Non-dioxin-like PCBs[b]	4.8	0.5	0.0
∑Mono-*ortho* PCBs[b]	5.3	2.0	0.0
∑Dioxin-like PCBs[b]	0.0	0.0	0.0
∑Cooke estrogenic PCBs[b]	2.6	0.0	0.0
∑Wolff estrogenic PCBs[b]	10.3	8.5	0.0
∑Cooke anti-estrogenic PCBs[b]	1.6	0.0	0.0
∑Wolff anti-estrogenic PCBs[b]	0.0	0.0	0.0

All estimates were adjusted for the survey design and sample weights.

[a]Cogeners not tested or >90% of results <LOD are not shown.

[b]Percent of participants with all congeners in sum <LOD.

4. Covariates

The potential confounders and effect modifiers evaluated in this investigation included age, study cycle, alcohol consumption, physical activity, BMI, family poverty-to-income ratio (PIR), race/ethnicity, CRP, cotinine, lipids, and use of certain medications. Both smoking and alcohol use are associated with an increased risk of osteoporosis (Hopper and Siman, 1994; Kanis et al., 2005) and smoking has been associated with lower body burdens of POPs (Jain and Wang, 2011). Serum cotinine was dichotomized as ≤10 ng/mL) and >10 ng/mL, a cutoff previously used as a marker for both active smoking and high environmental tobacco smoke exposure (Pirkle et al., 1996). Alcohol consumption was dichotomized as <12 drinks/year and ≥12 drinks/year. Exercise has beneficial effects on BMD in postmenopausal women (Dalsky et al., 1988; Nelson et al., 1994). We evaluated average level of physical activity per day categorized as an ordinal variable: low ("sits during the day and does not walk about very much"); medium ("stands or walks about a lot during the day, but does not have to carry or lift things very often"); or high ("lifts light loads or has to climb stairs or hills often or does heavy work [or] carries heavy loads") (CDC, 2014). Increased body weight is associated with decreased risk of osteoporosis. For 11 participants missing BMI data, we imputed BMI using height and weight obtained from self-reported weight history data. Body mass index was evaluated as a continuous variable. Because higher levels of CRP have been associated with lower BMD (de Pablo et al., 2012), we evaluated CRP concentration as a continuous variable. Clinical cutoffs were evaluated for BMI (<30 kg/m^2 and ≥30 kg/m^2) and CRP (<1 mg/dL and ≥1 mg/dL), but these variables did not add to the results. Poverty income ratio is a ratio of family income to the federal poverty threshold adjusted for family size. For 63 of the 603 participants who were missing PIR data, we imputed median PIR using values obtained from each survey cycle. African Americans have a higher BMD than white people, and African Americans have lower fracture rates at many skeletal sites (Looker et al., 2012). Participants were classified as Caucasian, African American, or Other. Serum lipids are generally associated with serum POP concentrations. Total serum lipids were calculated using the formula: lipids=[total cholesterol (mg/dL) x 2.27] + triglycerides (mg/dL) + 62.3 (CDC, 2006). Finally, we evaluated several medications that can affect bone metabolism (Rosen and Drezner, 2013), including anticonvulsants, antidiabetics, antirheumatic drugs, bisphosphonates, calcitonin, heparin, proton pump inhibitors, statins, thiazide diuretics, and thyroid hormones. Medications used in the past 30 days were identified using the Multum Lexicon Therapeutic Classification Scheme in the Prescription Medications-Drug Information questionnaire.

5. Statistical analyses

Statistical analyses were performed with SAS®-Callable SUDAAN® 11.0 (Research Triangle Institute, Research Triangle Park, North Carolina) using a calculated six-year sample weight provided by NHANES for the dioxins subsample and nest variables that accounted for the complex survey design. We followed NHANES guidelines for analyses of multiple imputation DXA data, which have been described elsewhere (CDC, 2008).

Natural log (ln) transformations of exposure variables and CRP were used to approximate a normal distribution with geometric means presented for descriptive purposes. For univariate analyses, we calculated means with PROC DESCRIPT; differences among study cycles were tested using PROC REGRESS. Categorical data were evaluated with PROC DESCRIPT, and differences between study cycles were tested using PROC MULTILOG. We used PROC REGRESS or PROC MULTILOG to calculate beta coefficients for bivariate analyses. We used multiple linear regression analyses to examine the effect of POPs on BMD using PROC REGRESS. *P*<.05 was considered statistically significant.

After merging the study cycles, ∑TEQs, ∑PCBs, PCB groupings, and individual PCB congeners (138, 153, and 180) were ranked into quintiles. Because a large percentage of the congeners <LOD occurred in the lower ranks, we combined the lowest two quintiles. We estimated dose-response models using indicator variables for quintiles 3, 4, and 5, with quintiles 1 and 2 pooled as the reference category. The ordinal exposure variable was used to test for linear trend across the categories. Sample weights (wet weight) of PCB, PCDD, and PCDF congeners instead of lipid-standardized measurements were used for all regression analyses (Schisterman et al., 2005). We decided a priori to include age, BMI, lipids, PIR, race, and study cycle as covariates in all multiple linear regression models. Confounding and effect modification were evaluated using ordinal exposure variables. To evaluate confounding, additional covariates were added individually to the adjusted model. Confounding was defined as a change in the exposure beta coefficient of more than 10%, on average, for the five BMD sites. Models containing ∑TEQs were further adjusted for bisphosphonates and thiazide diuretics and models containing ∑PCBs, ∑dioxin-like PCBs, ∑mono-*ortho* PCBs, and ∑Wolff anti-estrogenic PCBs were further adjusted for cotinine level. Effect modification was assessed using variables indicating the product of the potential effect modifier with the exposure in hierarchical regression models. When significant or borderline significant interaction (p<.10) was found, conditional effects were estimated and reported.

B. Results

1. Descriptive statistics

Table III summarizes continuous characteristics for postmenopausal participants as well as differences among study cycles. The mean age was 65 years (range 40–85+ years) and mean BMI was 29 kg/m^2 (range: 16.0–50.8 kg/m^2) (range data not shown). Congener classifications including ∑Mono-*ortho* PCBs, ∑dioxin-like PCBs, ∑Cooke anti-estrogenic PCBs, and ∑Wolff anti-estrogenic PCBs generally declined during the study cycles, while PIR, arm, leg, and pelvis BMD increased during the study cycles (p<.05).

Table IV summarizes categorical characteristics for postmenopausal participants as well as differences between study cycles. Thirty-eight percent of participants were classified as obese (BMI ≥30 kg/m^2) according to categories set by CDC (CDC, 2011) (data not shown), 19% had elevated cotinine levels, and medication use varied, with 13% using antidiabetics, 4% using bisphosphonates, 12.9% using thyroid hormones, and 9.4% using thiazide diuretics (Table IV). Less than 2% of study participants used antirheumatics, calcitonin, heparins, or thiazolidinediones (TZDs) (data not shown). The proportion of participants taking bisphosphonates significantly increased during the study cycles (p<.05).

TABLE III
CONTINUOUS CHARACTERISTICS FOR 603 ELIGIBLE POSTMENOPAUSAL WOMEN, NHANES 1999–2004

Characteristic	n	Combined	1999–2000 cycle	2001–2002 cycle	2003–2004 cycle	*P*-value[a]
		Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	
Ln∑TEQs (pg/g)[b]	524	0.15 (0.13–0.16)	0.14 (0.12–0.16)	0.18 (0.15–0.22)	0.14 (0.12–0.15)	0.85
Ln∑PCBs (ng/g)[b]	539	1.8 (1.7–1.9)	1.8 (1.6–2.0)	2.1 (1.9–2.4)	1.6 (1.4–1.8)	0.13
Ln∑Non-dioxin-like PCBs (ng/g)[b]	593	1.5 (1.4–1.6)	1.5 (1.3–1.6)	1.7 (1.5–1.9)	1.4 (1.3–1.5)	0.25
Ln∑Mono-*ortho* PCBs (ng/g)[b]	597	0.25 (0.23–0.28)	0.29 (0.25–0.34)	0.28 (0.25–0.32)	0.21 (0.19–0.24)	**0.0041**
Ln∑Dioxin-like PCBs (ng/g)[b]	543	0.26 (0.24–0.28)	0.28 (0.25–0.33)	0.31 (0.27–0.35)	0.21 (0.19–0.24)	**0.0048**
Ln∑Cooke estrogenic PCBs (ng/g)[b]	603	0.45 (0.42–0.48)	0.45 (0.40–0.52)	0.52 (0.46–0.58)	0.41 (0.37–0.44)	0.15
Ln∑Wolff estrogenic PCBs (ng/g)[b]	591	0.11 (0.11–0.12)	0.11 (0.10–0.12)	0.12 (0.11–0.14)	0.10 (0.09–0.11)	0.13
Ln∑Cooke anti-estrogenic PCBs (ng/g)[b]	546	0.09 (0.09–0.10)	0.10 (0.09–0.11)	0.11 (0.10–0.12)	0.08 (0.07–0.09)	**0.02**
Ln∑Wolff anti-estrogenic PCBs (ng/g)[b]	543	0.40 (0.37–0.44)	0.44 (0.39–0.52)	0.49 (0.44–0.54)	0.32 (0.29–0.38)	**0.0031**
LnPCB 138 (ng/g)[b]	602	0.27 (0.25–0.29)	0.24 (0.21–0.28)	0.31 (0.27–0.35)	0.26 (0.24–0.29)	0.39
LnPCB 153 (ng/g)[b]	601	0.38 (0.36–0.41)	0.39 (0.35–0.44)	0.43 (0.39–0.48)	0.34 (0.32–0.38)	0.09
LnPCB 180 (ng/g)[b]	596	0.28 (0.26–0.29)	0.27 (0.24–0.29)	0.30 (0.27–0.33)	0.27 (0.25–0.30)	0.86
Left arm BMD (g/cm^2)	603	0.67 (0.66–0.67)	0.62 (0.64–0.66)	0.66 (0.65–0.67)	0.69 (0.67–0.70)	**0.0002**[c]
Left leg BMD (g/cm^2)	603	1.02 (1.01–1.04)	0.98 (0.97–1.00)	1.01 (0.99–1.04)	1.07 (1.05–1.09)	**0.0001**[c]
Thoracic spine BMD (g/cm^2)	603	0.79 (0.78–0.80)	0.78 (0.76–0.81)	0.79 (0.77–0.81)	0.80 (0.77–0.82)	0.41
Lumbar spine BMD (g/cm^2)	603	0.95 (0.93–0.97)	0.94 (0.91–0.98)	0.94 (0.91–0.97)	0.96 (0.93–0.99)	0.40
Pelvis BMD (g/cm^2)	603	1.12 (1.10–1.14)	1.10 (1.08–1.13)	1.11 (1.08–1.14)	1.15 (1.12–1.18)	**0.03**[c]
Age (years)	603	65.4 (64.2–66.6)	66.7 (64.2–69.2)	65.1 (63.2–67.0)	64.7 (63.0–66.3)	0.19
BMI (kg/m^2)	603	28.9 (28.2–29.6)	28.9 (27.7–30.0)	28.6 (27.3–29.8)	29.2 (28.0–30.4)	0.65
Lipids (mg/dL)	603	710.0 (696.5–723.5)	713.2 (691.6–734.8)	687.6 (668.7–706.5)	726.4 (703.0–749.9)	0.37
LnCRP (mg/dL)[b]	603	0.28 (0.25–0.32)	0.32 (0.25–0.40)	0.27 (0.22–0.33)	0.26 (0.23–0.30)	0.15
Family PIR	603	2.5 (2.4–2.7)	2.2 (1.9–2.5)	2.5 (2.3–2.8)	2.8 (2.5–3.1)	**0.0066**

All estimates were adjusted for the survey design and sample weights.
[a]*P*-values in bold are statistically significant at $p<.05$.
[b]Geometric means are shown.
[c]Associations between BMD and study cycle remained significant after controlling for bisphosphonate use.
CI=Confidence interval

TABLE IV

CATEGORICAL CHARACTERISTICS FOR 603 ELIGIBLE POSTMENOPAUSAL WOMEN, NHANES 1999–2004

		Percent of total				
Characteristic		**Combined study cycles**	**1999–2000**	**2001–2002**	**2002–2004**	***P*-value[a]**
Sample size		**603**	**194**	**203**	**206**	
Age	40–50 years	9.1	6.8	10.4	9.9	**0.0056**
	51–60 years	26.0	20.5	27.8	29.0	
	61–70 years	31.7	32.3	30.1	32.7	
	71–80 years	21.6	29.6	18.8	17.2	
	81–85+ years	11.6	10.8	13.0	11.1	
Alcohol consumption	≥12 drinks/year	50.4	49.8	52.2	49.3	0.94
Cotinine >10 ng/ml		19.2	21.3	23.0	14.2	0.12
Physical activity/day	Low	26.6	32.0	22.9	25.3	0.39
	Medium	62.1	56.5	64.4	64.8	
	High	11.3	11.5	12.7	9.9	
Race/ethnicity	Caucasian	72.8	68.0	72.5	77.0	0.47
	African American	11.0	11.5	12.0	9.6	
	Other	16.3	20.5	15.5	13.4	
Anticonvulsants		5.1	4.1	6.0	5.2	0.77
Antidiabetics		13.2	15.9	9.5	14.1	0.73
Bisphosphonates		4.3	1.6	2.5	8.2	**0.0024**
Proton pump inhibitors		8.9	8.0	7.0	11.2	0.46
Statins		16.6	12.8	19.6	17.2	0.45
Thyroid hormones		12.9	10.9	13.9	13.8	0.55
Thiazide diuretics		9.4	7.3	8.6	11.9	0.26
Bilateral oophorectomy		22.0	23.0	13.8	28.1	0.34
Hysterectomy		37.2	39.1	32.3	39.7	0.85

All estimates were adjusted for the survey design and sample weights.

[a]*P*-values in bold are statistically significant at $p<.05$.

2. Bivariate analyses

For bivariate analyses (data not shown), we selected ∑mono-*ortho* PCBs, ∑Cooke estrogenic PCBs, and PCB 138 as representative exposures of interest. In general, significant positive associations were found for ∑mono-*ortho* PCBs, ∑Cooke estrogenic PCBs, and PCB 138 with age, lipids, African American race (compared with Caucasian race), and thiazide diuretics. Cotinine level and PIR were significantly and inversely associated with exposures. In bivariate analyses with BMD measures, we generally found significant positive associations with BMI, PIR, CRP, and African American race (compared with Caucasian race), while age and bisphosphonate use were significantly and negatively associated with BMD. The associations between BMD with CRP and BMD with bisphosphonates were no longer significant after adjusting for age, BMI, race, study cycle, and PIR.

3. Multiple linear regression models

Table V shows dose-response models for exposures in quartiles. In adjusted models, we found a positive monotonic dose-response (trend *p*-value <0.05) for the associations of ∑mono-*ortho* PCBs with leg BMD, ∑Cooke estrogenic PCBs with lumbar spine BMD, and PCB 138 with both thoracic and lumbar spine BMD. No other significant monotonic dose-response associations were found; however, several associations were of borderline significance (.05 < trend *p*-value <.10). In addition, several associations showed a U-shaped dose-response pattern (.05 < trend *p*-value <.10). Overall, stronger and significant or borderline significant associations were generally observed for quintile 5 versus quintiles 1–2.

TABLE V

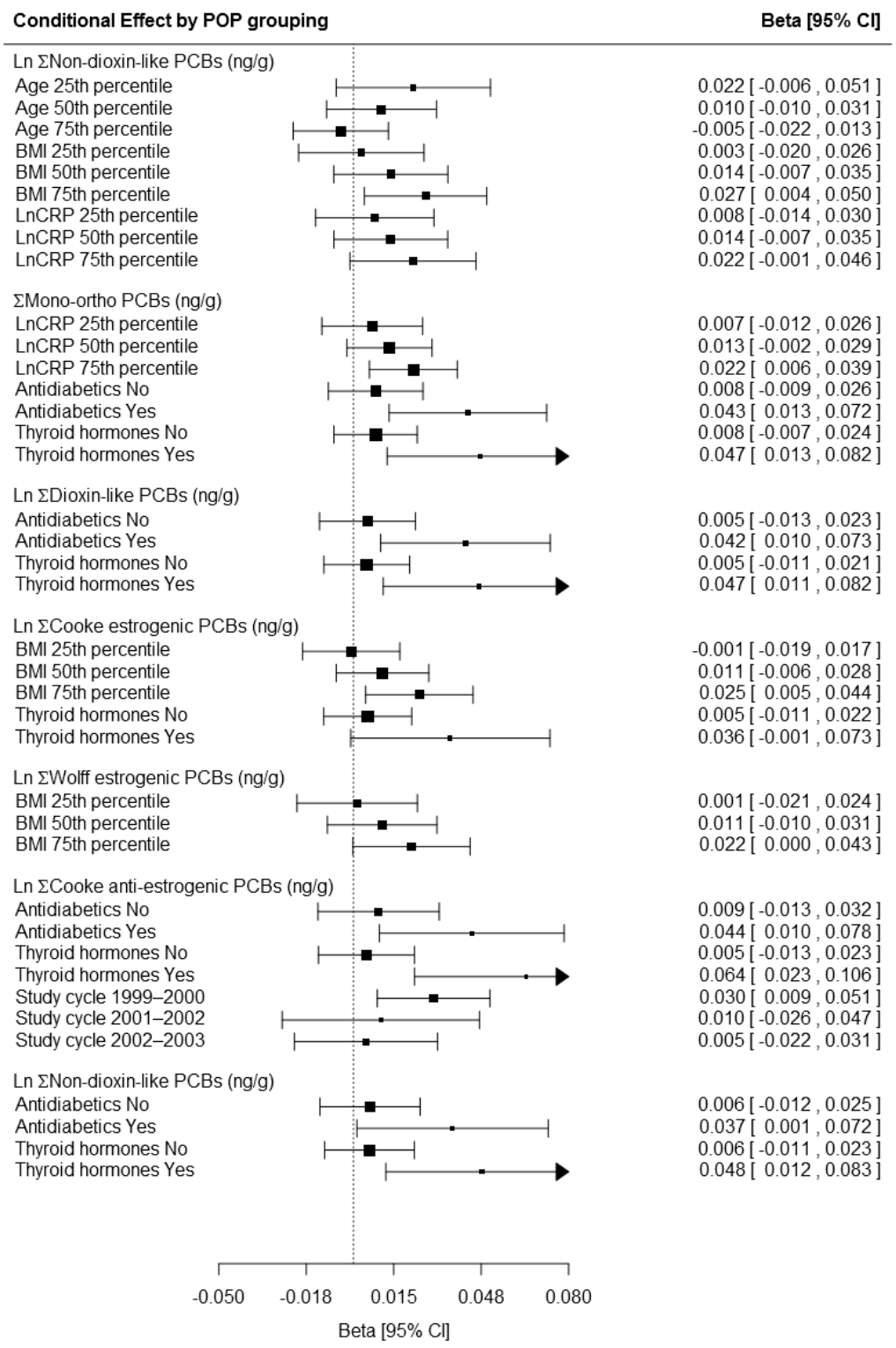
ASSOCIATIONS OF POPS IN QUARTILES WITH BMD IN 603 ELIGIBLE POSTMENOPAUSAL WOMEN, NHANES 1999–2004

POP, BMD site	Quartile 1–2	Beta Quartile 3	Beta Quartile 4	Beta Quartile 5	Trend *p*-value[a]
Ln∑TEQs (pg/g)[b]					
Left leg BMD (g/cm^2)		0.03438	0.027864	0.032701	0.05
p-value[c]		**0.02**	0.10	0.07	
Ln∑Non-dioxin-like PCBs (ng/g)[d]					
Left leg BMD (g/cm^2)		0.012070	0.016841	0.038706	0.09
p-value		0.49	0.29	0.09	
Thoracic spine BMD (g/cm^2)		0.010378	0.007499	0.035623	0.07
p-value[c]		0.53	0.63	**0.04**	
Lumbar spine BMD (g/cm^2)		0.024115	0.021591	0.045311	0.08
p-value		0.31	0.34	0.08	
Ln∑Mono-*ortho* PCBs (ng/g)[e]					
Left leg BMD (g/cm^2)		-0.003045	0.012460	0.035575	**0.049**
p-value[c]		0.78	0.32	**0.046**	
Ln∑Dioxin-like PCBs (ng/g)[e]					

TABLE V (continued)

POP, BMD site	Quartile 1–2	Beta Quartile 3	Beta Quartile 4	Beta Quartile 5	Trend *p*-value[a]
Left leg BMD (g/cm^2)		-0.004830	0.013486	0.031864	0.07
p-value		0.66	0.30	0.09	
Ln∑Cooke estrogenic PCBs (ng/g)[d]					
Left leg BMD (g/cm^2)		0.011431	0.021014	0.035505	0.05
p-value		0.39	0.22	0.05	
Thoracic spine BMD (g/cm^2)		0.007620	0.014128	0.031357	0.06
p-value		0.62	0.36	0.06	
Lumbar spine BMD (g/cm^2)		0.018255	0.022161	0.053154	**0.049**
p-value[c]		0.45	0.31	**0.049**	
Ln∑Wolff estrogenic PCBs (ng/g)[d]					
Lumbar spine BMD (g/cm^2)		0.029019	0.037872	0.045755	0.05
p-value		0.10	0.12	0.06	
Ln∑Cooke anti-estrogenic PCBs (ng/g)[d]					
Left leg BMD (g/cm^2)		0.008663	0.023861	0.035265	0.07
p-value		0.55	0.10	0.10	
Thoracic spine BMD (g/cm^2)		0.001653	0.021273	0.028519	0.07
p-value		0.92	0.25	0.10	
LnPCB 138 (ng/g)[b]					
Thoracic spine BMD (g/cm^2)		0.014404	0.020665	0.035878	**0.01**
p-value[c]		0.33	0.16	**0.01**	
Lumbar spine BMD (g/cm^2)		0.024993	0.037686	0.053185	**0.02**
p-value[c]		0.20	0.11	**0.03**	
LnPCB 153 (ng/g)[d]					
Left leg BMD (g/cm^2)		0.018688	0.026375	0.038396	0.05
p-value		0.17	0.098	0.07	
Thoracic spine BMD (g/cm^2)		0.020403	0.012773	0.036929	0.05
p-value[c]		0.18	0.43	**0.03**	
Lumbar spine BMD (g/cm^2)		0.024108	0.022931	0.050617	0.06
p-value		0.28	0.33	0.05	
LnPCB 180 (ng/g)[d]					
Left leg BMD (g/cm^2)		0.023108	0.034619	0.041104	0.05
p-value[c]		0.08	**0.04**	0.09	
Thoracic spine BMD (g/cm^2)		0.018713	0.016836	0.034577	0.09
p-value		0.25	0.37	0.05	

All estimates were adjusted for the survey design and sample weights.
[a]Trend *p*-values were obtained using an ordinal variable for exposures.
[b]Multiple linear regression models were adjusted for age, BMI, lipids, race, PIR, survey cycle, bisphosphonates, and thiazide diuretics.
[c]*P*-values in bold are statistically significant at $p<.05$.
[d]Multiple linear regression models were adjusted for age, BMI, lipids, race, PIR, and survey cycle.
[e]Multiple linear regression models were adjusted for age, BMI, lipids, race, PIR, survey cycle, and cotinine.



Box size indicates the precision of the estimate; larger boxes signify less variance.
A confidence interval (CI) that extends beyond the plot domain is indicated by an arrow.

Figure 1. Selected associations of POPs with pelvis BMD by potential effect modifiers in 603 eligible postmenopausal women, NHANES 1999–2004.

4. Effect modification

Statistically significant interactions were most commonly observed for the arm, leg, and pelvis. Table VI, Appendix A shows *p*-values for interactions of potential effect modifiers and POPs. To help understand the pattern of interaction, we displayed estimates centered at the 25th, 50th, and 75th percentiles of continuous potential effect modifiers. In general, we saw the strongest ordinal dose-response for BMI and CRP centered at the 75th percentile, whereas the strongest ordinal dose-response for age was at the 25th percentile. In addition, Table VI, Appendix A shows *p*-values for interactions of dichotomous variables and POPs. To help understand the pattern of interaction, we displayed estimates conditioned on dichotomous variable status. In general, we saw the strongest ordinal dose-response for thyroid hormone and antidiabetic medication use. As expected, BMI was strongly related to antidiabetic medication use in the present study (data not shown). When POPs were evaluated according to classification schemes, associations of estrogenic and/or non-dioxin-like PCB groupings with BMD depended on BMI, with stronger effects in obese participants. On the other hand, associations of anti-estrogenic and/or dioxin-like POP groupings with BMD depended on antidiabetic or thyroid hormone medication use, with stronger effects in postmenopausal women who used these medications in the past 30 days. Finally, we observed some effect modification of the association of individual PCB congeners with BMD by lipids, with stronger effects centered at the 75th percentile. Selected associations of POPs and pelvis BMD by potential effect modifiers are presented in Figure 1.

5. Sensitivity analyses

Lipid standardization of POPs, or the division of serum POP concentrations by serum lipids, was found to be subject to bias (Schisterman et al., 2005); therefore, we used wet weight POP measurements controlled for lipids in multiple regression models. When we repeated the analysis using lipid-standardized measurements, significant or borderline significant relationships between BMD and exposure measurements remained, except for the association between PCB 138 and thoracic spine BMD, which became nonsignificant. Because older postmenopausal women would have experienced longer duration of exposure and also higher PCB exposure levels in the past (Schecter et al., 2005), a sensitivity analysis for statistically significant associations was conducted for the effects of age centered at the 25th, 50th, and 75th percentile. Dose-response relationships of PCBs with BMD were generally significant or borderline significant at the 25th and 50th percentile; however, associations were no longer significant at the 75th percentile. Because several important study variables were significantly different across study cycles (Tables III and IV), statistically significant associations were conditioned on study cycle. The dose-response associations of POPs with BMD varied but were generally significant for the first study cycle and not statistically significant for the 2001–2002 and 2003–2004 study cycles. However, we found statistical evidence for effect modification by study cycle for ∑Cooke estrogenic PCBs and limited evidence for ∑dioxin-like PCBs. Bilateral oophorectomy may have adverse skeletal effects in postmenopausal women (Melton et al., 2003), although the literature has been inconsistent (Kritz-Silverstein et al., 2004);

therefore, we repeated the current analysis excluding postmenopausal participants with bilateral oophorectomy. This analysis yielded similar results. In addition, 12 participants reported the use of TZDs, which are a class of insulin-sensitizing agents used to treat diabetes. This class of medication may have adverse effects on bone (Okazaki et al., 1999; Kahn et al., 2008) and may also affect estrogen level as a result of inhibition of aromatase activity (Seto-Young et al., 2011). When we repeated the analysis excluding participants who used TZDs in the past 30 days, similar results were observed. Because a large percentage of the congeners <LOD occurred in the lower ranks, we combined the lowest two quintiles for exposure variables to compensate for lack of precision around the LOD. In order to evaluate model specification, we repeated the analysis using continuous log transformed exposure variables and found that models were somewhat biased away from the null. We also estimated dose-response models using indicator variables for quintiles 2, 3, 4, and 5, with quintile 1 as the reference category. Results were essentially unchanged.

C. Discussion

1. Bone mineral density

The current study found a dose-response increase in BMD with exposure to ∑Cooke estrogenic PCBs, ∑mono-*ortho* PCBs, and PCB 138. However, the most striking observation in this investigation is the demonstration of a significant positive relationship between serum POP levels and BMD in postmenopausal participants who were younger, obese, or diabetic; were taking thyroid hormones; or had elevated CRP levels, suggesting an interaction between endogenous estrogen status and POP exposure. When POPs were assessed according to classification schemes, associations of estrogenic and/or non-dioxin-like PCB groupings with BMD depended on BMI, with stronger effects in obese participants. Alternatively, associations of anti-estrogenic and/or dioxin-like POP groupings with BMD depended on antidiabetic or thyroid hormone medication use, with stronger effects in participants who used these medications in the past 30 days.

A recent investigation found no evidence that postnatal TCDD exposure adversely affects adult bone parameters (Eskenazi et al., 2013). To our knowledge, epidemiologic data on the effects of PCBs on BMD in adults are limited to five published studies with regression models minimally adjusted for age and BMI, height, or weight. First, PCBs grouped according to mechanism of action, including estrogenic and anti-estrogenic activity, were not significantly associated with BMD in 155 Swedish men from the general population (Glynn et al., 2000). Second, an investigation of 196 Baltic Coast fishermen and 184 spouses found no significant association of serum PCB 153 with BMD after adjustment for age and BMI (Wallin et al., 2005). Third, Hodgson et al. (2008) found significant negative associations of PCB 118 with BMD in 157 men living near the Baltic coast and a river contaminated with PCBs, but a significant positive association with the sum of PCB 138, 153, and 180. In 167 women, PCB 118 was significantly and positively associated with BMD. Fourth, PCB 153 was not significantly associated with

BMD in 908 postmenopausal Swedish women (Rignell-Hydbom et al., 2009). Fifth, among eight POPs, only PCB 126 was positively and significantly associated with BMD among 679 women and 612 men aged 50 years and older. When effect modification by FM was evaluated, "POPs were inversely associated with BMD among women with low FM while they were positively associated with BMD among women with high FM" (Cho et al., 2011). Our findings of a positive association of PCBs with BMD are consistent with Hodgson et al. (2008) and Cho et al. (2011). Overall, the results of epidemiologic studies on the effects of PCBs on BMD have been inconsistent. A lack of association between POPs and BMD in some investigations could be indicative of a threshold of bioavailable estrogen necessary for impacts on bone. Further, the effect of mixtures of PCBs, PCDDs, and PCDFs might be additive or antagonistic, depending on the population and dose.

The present study adds to the Cho et al. (2011) investigation of POPs and BMD in several ways. First, the evaluation of estrogenic and/or non-dioxin-like and anti-estrogenic and/or dioxin-like POP groupings may offer insight into potential biological pathways. We used exposures measured in quintiles and combined the lowest two quintiles to account for the varying percent below the LOD and followed NHANES guidelines for analyses of multiple imputation DXA data. In addition, we included a robust definition of menopause, evaluated medications that can affect bone as potential confounders or effect modifiers, and excluded postmenopausal women who took sex hormones or glucocorticoids.

The findings in our study are consistent with animal studies on the effect of POPs on BMD and other bone indicators. The effects appear to be modified by estrogen status. Investigations in rats exposed to the dioxin-like PCB 126 showed impaired bone strength and composition, and effects were modulated depending on the estrogen profile of the animal. In sham-operated rats, PCB 126 exposure was not related to BMD or trabecular bone volume of the tibia, whereas in ovariectomized rats, PCB 126 exposure resulted in a decreased length and increased tibial BMD (Lind et al., 1999). Moreover, estrogen supplementation in rats exposed to PCB 126 modulated effects on bone tissue. In ovariectomized rats, estradiol supplementation modulated PCB 126-related effects resulting in increased trabecular bone volume; however, the opposite was found in sham-operated rats (Lind et al., 2004). Further, estrogenic and anti-estrogenic aroclors were found to differentially affect bone metabolism, mainly in ovariectomized rats (Yilmaz et al., 2006).

2. <u>Effect modification</u>

In the present study, associations of POPs with BMD were stronger in younger postmenopausal women. Bone is a target tissue for estrogen and estrogen deficiency and is known to play an important role in the osteoporotic process (D'Amelio et al., 2008). Previous literature supports the threshold estradiol hypothesis for skeletal sufficiency (Riggs et al., 2002; Mellstrom et al., 2008; Gennari et al., 2011). "The main source of estrogen after menopause is from the aromatization of

androgenic precursors, a reaction that is catalyzed by the aromatase enzyme and predominantly occurs in the adipose tissue, with small amounts also produced in bone" (Mullin et al., 2011). During the transition to menopause, serum estradiol levels do not begin to decline until less than a year before menopause (Rannevik et al., 1995). The average circulating estradiol levels in perimenopausal women are estimated to be somewhat higher than those in younger women because of an increased follicular response to elevated FSH (Santoro et al., 1996). In addition, there is preliminary evidence in 23- to 61-year-olds that the expression of aromatase, which is responsible for estrogen biosynthesis, increases in fat with advancing age (Bulun and Simpson, 1994; Agarwal et al., 1997). However, levels of the circulating c19 steroid precursors, which are essential substrates for peripheral estrogen synthesis, have been shown to decline markedly with age up to 80 years (Labrie et al., 1997). Sex hormone-binding globulin decreases from the age of 20 to approximately 60 years and then progressively increases at older ages (Maggio et al., 2008), with differences in insulin levels (Preziosi et al., 1993) or BMI (Haffner et al., 1991) potentially contributing to SHBG regulation.

Associations of POPs with BMD were stronger in postmenopausal women with elevated CRP levels. There are several studies noting associations of CRP with factors relating to estrogen status, including higher levels of estradiol and lower levels of SHBG (Stork et al., 2008; Maggio et al., 2011), as well as stimulation of aromatase (Zhao et al., 1996). Some studies have suggested that PCBs may act through inflammation (Hennig et al., 2002). In the current study, CRP was not associated with antidiabetic medications but was associated with BMI and triglycerides after adjusting for potential confounders (data not shown).

Associations of estrogenic and/or non-dioxin-like PCB groupings with BMD in the present study were stronger in obese postmenopausal participants. There is evidence that body composition may play an important role in steroid hormone and SHBG concentrations. In general, the aromatization of androstenedione to estrone in adipose tissue correlates positively with weight (Bulun et al., 1994). Additionally, inverse correlations have been reported between BMI and SHBG for postmenopausal women, resulting in increased bioavailable estradiol (Cauley et al., 1989).

Findings of stronger associations of anti-estrogenic and/or dioxin-like POP groupings with BMD among postmenopausal women taking antidiabetic or thyroid hormone medications are novel. There is evidence that androgens have effects on bone physiology, with androgen deficiency resulting in decreased bone density (Yasui et al., 2012). Systemic hormones involved in stimulating bone formation may also include insulin, and increased BMD in those with diabetes may be due in part to greater obesity associated with the disease (Haffner and Bauer, 1993; Christensen and Svendsen, 1999). In fact, "Older white women with type 2 diabetes have higher areal BMD than women without diabetes, even after adjusting for body

size" (Strotmeyer et al., 2004). In addition, the metabolism of both androgens and estrogens is altered in hypothyroidism. Androgen production is decreased, and the metabolism of testosterone is shifted toward etiocholanolone rather than androsterone. "With respect to estradiol and estrone, hypothyroidism favors metabolism of these steroids via 16α-hydroxylation over 2-oxygenation, resulting in increased formation of estriol at the expense of 2-hydroxyestrone and its derivative, 2-methohyestrone" (Doufas and Mastorakos, 2000). The SHBG in plasma is decreased, resulting in decreased plasma concentrations of both testosterone and estradiol, but the unbound fractions are increased (Brenta et al., 1999).

In the present study, we found that associations of estrogenic and/or non-dioxin-like PCB groupings with BMD depended on BMI, while associations of anti-estrogenic and/or dioxin-like POP groupings with BMD depended on antidiabetic or thyroid hormone medication use. Notably, a majority of congeners contributing to the anti-estrogenic groupings in this study have been classified as dioxin-like. The observed effect modification of associations between POP classification schemes and increased BMD may be attributed to the fact that "dioxin-like compounds and [non-dioxin-like] PCBs do not exert a similar pattern of toxicological effects essentially because of differences in their mechanism of action" (Heilier et al., 2008). Dioxin-like compounds exert their anti-estrogenic effects through interaction with AhR; however, dioxins have been related to endometriosis and estrogen-dependent tumors (van Larebeke et al., 2001). Further, some investigators have hypothesized that dioxin-like compounds may exert estrogenic effects resulting from cross-talk between the estrogen and the AhR in the regulation of various signaling pathways (Ohtake et al., 2003).

3. Limitations and strengths

The present investigation has a number of limitations. The cross-sectional design of the study does not allow us to establish a temporal relationship of POP exposures with BMD changes. Because obesity was found to modify the association of POP exposures with BMD, adjusting for BMI in multiple linear regression analyses may not be appropriate. We adjusted for many factors including medications and cotinine level that could affect the relationship between PCBs and BMD, but there were many concurrent exposures, such as p,p′-diphenyldichloroethene or other organochlorine pesticides, for which we have not controlled. Additional congener measurements for complete congener groupings might have helped to better elucidate mechanisms related to associations between PCB groupings and BMD. Further, estrogenic and anti-estrogenic classes of PCB congeners can express varied and sometimes conflicting effects (Warner et al., 2012). Associations were generally significant for the 1999–2000 study cycle but not the 2001–2002 and 2003–2004 study cycles. In addition, the 2003–2004 study cycle did not have FSH measurements for the identification and exclusion of potentially misclassified postmenopausal women <60 years of age. Finally, the findings might be due to chance, as multiple comparisons were made in the statistical analysis. We did not adjust for multiple comparisons; however, the purpose of these exploratory analyses was to inform and guide future

research that could be subject to further rigorous testing (Goldberg et al., 2011). Despite the limitations, our investigation has a number of strengths, including a large and representative sample of the US population; consistency with findings of some studies; and multiple BMD sites, exposures groupings, and confounders measured. Using conditional analysis, we centered potential effect modifiers to ensure we did not limit the power of our analysis.

D. Conclusion

In this investigation, we found few significant positive associations of POPs with BMD in postmenopausal women. Significant positive relationships were observed between serum POP levels and BMD in postmenopausal participants who were younger, obese, or diabetic; were taking thyroid hormones; or had elevated CRP levels, suggesting an interaction between endogenous estrogen status and POP exposure. Future studies should investigate the mechanisms underlying the observed interactions.

IV. ASSOCIATIONS OF PERSISTENT ORGANIC POLLUTANTS WITH FOLLICLE-STIMULATING HORMONE AND LUTEINIZING HORMONE IN POSTMENOPAUSAL WOMEN: NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY 1999–2002

Polychlorinated biphenyls are a class of heat-resistant, oily liquids that were used as insulating fluids in capacitors and transformers (ATSDR, 2000). Polychlorinated dibenzo-p-dioxins and PCDFs, commonly known as dioxins, are compounds formed as unintentional by-products of industrial processes (Fiedler, 1996). Like PCBs, dioxins are resistant to abiotic and biotic degradation in the environment and bioaccumulate and magnify in animals and humans (Safe, 1994; Van den Berg et al., 1998). Human body burdens of these chemicals have declined over time (Aylward et al., 2002; Turyk et al., 2012); however, continued exposure is principally through consumption of contaminated food items (van Larebeke et al., 2001).

Congeners of PCBs, PCDDs, and PCDFs have varying patterns of toxicity. The AhR has a high affinity for 2,3,7,8-substituted PCDDs and PCDFs in addition to some non- and mono-*ortho* substituted PCBs (Poland et al., 1985; Safe et al., 1985; Van den Berg et al., 1998). Although AhR-mediated biochemical and toxic responses are generally considered to be anti-estrogenic, dioxins have been shown to induce endometriosis and estrogen-dependent tumors (van Larebeke et al., 2001), suggesting cross-talk between estrogen receptors and the AhR (Ohtake et al., 2003). In addition, dioxin-like, potentially anti-estrogenic PCBs were found to up-regulate the gene expression of receptors and enzymes involved in steroid metabolism, including estrogen receptor beta and CYP19, which encodes aromatase, the enzyme known to convert androgen precursors to estrogen (Warner et al., 2012). Some PCBs may also exhibit estrogenic effects by inhibiting estrogen sulfotransferase, the enzyme that inhibits estrogen metabolism (Kester et al., 2000; Kester et al., 2002). Recently, it has been suggested that dioxins and dioxin-like POPs up-regulate genes related to body fat, insulin resistance, and inflammation (Kim et al., 2012). Whether any of these mechanisms are relevant for POP effects on gonadotropin homeostasis is presently unknown. Finally, PCBs were found to act directly on gonadotropin-releasing hormone gene expression, suggesting hypothalamic level effects (Gore et al., 2002).

Persistent organic pollutants have been shown to exhibit a broad range of toxic effects including disruption of steroid hormone homeostasis. Gonadotropin hormones are regulated by the HPG axis. Reduction of circulating estradiol after menopause is compensated for by release of FSH and LH from the pituitary. In cycling women, the increase in FSH and LH in turn stimulates the ovaries to produce more estradiol, whereas after menopause, circulating levels of estradiol are derived from aromatization of androgens to estrogens in adipose tissue. Overweight women convert more androgens to estrogens when compared with women of normal weight due to increased aromatase activity. The bioavailability of estradiol in circulation is affected by proteins known as SHBG. Estradiol is transported to peripheral tissues primarily bound to SHBG as a complex known as SHBG-E2, but estradiol can also be loosely bound to albumin. Synthesis of SHBG by the liver is affected by

the estrogen-androgen balance, with estrogens stimulating and androgens suppressing their production (Bulun et al., 2008). Other factors related to health may alter SHBG levels, including hypothyroidism, obesity, and inflammation, and, in turn, affect the bioavailability of estradiol.

Studies in male and female animals and in vitro investigations have shown associations between PCB exposure and FSH and LH levels, although results have been inconsistent and differed by PCB congener or aroclor (Desaulniers et al., 1999; Wade et al., 2002; Oskam et al., 2005; Uslu et al., 2013). To date, there is only one known study in occupationally exposed postmenopausal women that has found an inverse association between PCBs and FSH and SHBG (Persky et al., 2011). It is well established that men and women have different hormone profiles. In men occupationally exposed to TCDD, positive relationships were observed with FSH and LH (Sweeney et al., 1997). Several investigations in men, however, did not observe relationships between POPs and LH or FSH (Persky et al., 2001; Rignell-Hydbom et al., 2004; Turyk et al., 2006).

In this investigation, we explored the association of POP exposure with FSH and LH levels in postmenopausal women using the NHANES, a cross-sectional survey examining a random sample of the US population (CDC, 2014). The present study uses PCB, PCDD, PCDF, FSH, and LH measurements obtained in the 1999–2000 and 2001–2002 survey cycles. We examined the effects of general population POP exposures on FSH and LH in a subgroup of postmenopausal women not taking glucocorticoids or sex hormones. This report is focused on the linear relationships of FSH and LH with dioxin-like TEQs, individual PCB congeners, ∑PCBs, and PCB congeners grouped into categories with similar structure or a common mechanism of action. In addition, we examined the hypothesis that associations of POPs with FSH and LH are modified by factors that may influence endogenous hormones.

A. Methods

1. Participants

Data from the NHANES survey cycles conducted in 1999–2000 and 2001–2002 were obtained online (CDC, 2014). Each survey is a nationally representative sample of the US civilian, noninstitutionalized population based on a complex probability sampling design. Any associations between POPs and gonadotropins may be more clearly observed among postmenopausal women who are no longer cycling and, in turn, under less influence of ovarian estrogen. Menopause is defined as one year after the permanent cessation of menstrual periods, which women experience at the average age of 51 years. Menopause can occur naturally or be induced through a medical intervention such as bilateral oophorectomy. Normal FSH levels for premenopausal women are 4.7–21.5 mIU/mL, while normal FSH levels for postmenopausal women are 25.8–134.8 mIU/mL (Lobo, 2007). Standards used to define menopause were based on a prior report (Kalkwarf et al., 2003) and were

applied consecutively so that the rules were applied only to women not already in a previous category. The following are inclusion categories for postmenopausal women:

1. Any age and last period ≥12 months without hysterectomy or with hysterectomy and bilateral oophorectomy
2. 56–59 years of age and last period ≥12 months with hysterectomy and without bilateral oophorectomy, and FSH ≥25.8 mIU/mL
3. <56 years of age and last period ≥12 months with hysterectomy, without bilateral oophorectomy, and FSH ≥50 mIU/mL

In the present investigation, we focused on the 1,847 postmenopausal women ≥40 years of age with questionnaire data that included hysterectomy, bilateral oophorectomy, and medication. We excluded postmenopausal participants who were <40 years of age because they were assumed to have experienced early menopause (*n*=3). Participants were excluded if they did not have exposure or hormone measures (*n*=1,697). Serum levels of gonadotropins were originally used by NHANES investigators to classify women according to menopausal status; therefore, FSH and LH tests were performed only on women aged 35–60 years. Complete data for analysis of the associations of POPs with FSH and LH were available for 89 participants after excluding participants <60 years of age with FSH <25.8 mIU/mL (*n*=10) and with missing cotinine data (*n*=1). We also excluded participants who reported taking glucocorticoids (*n*=3) and who specified taking sex hormones (estrogen, progestins, sex hormone combinations, miscellaneous sex hormones, gonadotropin-releasing hormones and analogs, androgens and anabolic steroids, and contraceptives) or other hormones/hormone modifiers, including SERMs, aromatase inhibitors, antiandrogens, and antigonadotropic agents (*n*=44).

2. <u>Follicle-stimulating hormone, luteinizing hormone, and other physiological measurements</u>

Details of the NHANES laboratory measurements are available online for 1999–2000 (CDC, 2010a) and 2001–2002 (CDC, 2010b). Briefly, microparticle enzyme immunoassay technology was used to measure serum FSH and LH concentrations for 1999–2000. The sensitivity for FSH was 0.2 IU/L and the sensitivity for LH was 0.5 IU/L. Serum FSH and LH concentrations for 2001–2002 were measured by a paramagnetic particle, chemiluminescent two-step enzyme assay. The sensitivity for FSH and LH was 0.02 IU/L. Measurements below the LOD were imputed as 0.2 or 0.5 IU/L divided by the square root of two by the CDC. The laboratory assay coefficient of variation (CV) for 1999–2000 varied from 2.37 to 7.95 for FSH and from 1.65 to 7.59 for LH (CDC, 2010a). The CV for 2001–2002 varied from 3.2 to 7.2 for FSH and from 3.3 to 10.1 for LH (CDC, 2010b). The CV was dependent on the lot sampled. Levels of CRP were measured by latex-enhanced nephelometry with high sensitivity by using a Dade Behring Nephelometer II Analyzer System (Dade Behring Diagnostics, Inc., Somerville, New Jersey).

Serum total cholesterol was measured enzymatically after hydrolyzation and oxidation, while triglycerides were analyzed enzymatically after hydrolyzation into glycerol. Concentration of GGT was assayed using a Hitachi 737 Analyzer (Boehringer-Mannheim Diagnostics, Indianapolis, Indiana. Serum cotinine was measured using isotope dilution high-performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry. No LOD was reported.

3. Polychlorinated biphenyl, polychlorinated dibenzo-p-dioxin, and polychlorinated dibenzofuran measurements

All POPs were measured in serum by high-resolution gas chromatography/isotope-dilution high-resolution mass spectrometry (Organic Toxicology Branch, National Center for Environmental Health, CDC, Atlanta, Georgia). The various congener groupings used in the current study are listed in Table VII. Compounds able to bind to the AhR (PCDDs, PCDFs, and dioxin-like PCBs) were used to calculate ∑TEQs by summing "the products of the concentration of each compound multiplied by its [toxic equivalency factor] value" (Van den Berg et al., 2006). Congener-specific values for PCB congeners were summed to yield ∑PCBs. The PCB congeners were grouped according to structure including ∑non-dioxin-like PCBs, ∑mono-*ortho* PCBs, and ∑dioxin-like PCBs (consisting of non-*ortho* and mono-*ortho* PCBs), and were also grouped according to mechanism of action, including estrogenic and anti-estrogenic activity (Wolff et al., 1997; Cooke et al., 2001). Polychlorinated biphenyl 126 is listed in both ∑Cooke estrogenic PCBs and ∑Cooke anti-estrogenic PCBs. We also selected three PCB congeners (PCB 138, PCB 153, and PCB 180) for the analysis because they are often the most prevalent PCBs found in human serum (CDC, 2009). For congeners with results below the LOD, the measurement was imputed by CDC as the LOD for that specific congener divided by the square root of two. The LOD differed for each participant because it was based on sample volume. In the first study cycle, more of the individual congener measurements were below the LOD than in the second study cycle. Only congeners that had >10% of results >LOD for each of the two study cycles were included in the analysis. When results for more than one congener were not reported by CDC for a participant, the participant was coded as missing for each summary exposure mentioned previously. However, ∑Wolff estrogenic PCBs comprised two congeners; therefore, the participant was coded as missing if one of the congeners was not reported by CDC.

TABLE VII

CONGENER GROUPINGS, NHANES 1999–2002

Grouping	Congeners[a]
∑TEQs[b]	PCB congeners **105, 118, 126, 156, 169**; PCDD congeners **1,2,3,7,8-PentaPCDD, 1,2,3,6,7,8-HexaPCDD, 1,2,3,7,8,9-HexaPCDD, 1,2,3,4,6,7,8-HeptaPCDD, 1,2,3,4,6,7,8,9-OctaPCDD**; PCDF congeners **2,3,4,7,8-PentaPCDF, 1,2,3,4,7,8-HexaPCDF, 1,2,3,6,7,8-HexaPCDF, 1,2,3,4,6,7,8-HeptaPCDF**
∑PCBs[b]	**74, 99, 105, 118, 126, 138, 146, 153, 156, 169, 170, 177, 178, 180, 183, 187**
∑Non-dioxin-like PCBs[b]	**74, 99, 138, 146, 153, 170, 177, 178, 180, 183, 187**
∑Mono-*ortho* PCBs	**105**, 114, **118**, 123, **156**, 157, 167, 189
∑Dioxin-like PCBs	77, 81, **105**, 114, **118**, 123, **126**, **156**, 157, **169**, 189
∑Cooke estrogenic PCBs	1, 3, 4, 8, 15, 18, 21, 31, 44, 47, 48, 49, 52, 54, 61, 70, 75,77, 80, 95, **99**, 101, 104, 110, **126**, 136, **153**, 155, 184, 188
∑Wolff estrogenic PCBs	101, 174, **177**, **187**, 201
∑Cooke anti-estrogenic PCBs	37, 77, 81, **105**, 114, **126**[c], 155, **156**, **169**
∑Wolff anti-estrogenic PCBs	66, **74**, 77, **105**, **118**, **126**[c], 155, **156**, **169**

[a]Congeners in bold were included in the grouping.
[b]Only measured congeners in the grouping are shown.
[c]PCB 126 is listed in both ∑Cooke estrogenic PCBs and ∑Cooke anti-estrogenic PCBs.

4. Covariates

Potential confounders and effect modifiers evaluated in this study included age, alcohol consumption, BMI, CRP, cotinine level, family PIR, GGT, lipids, race/ethnicity, study cycle, antidiabetic medications, and thyroid hormone medications. Alcohol consumption was dichotomized as <12 drinks/year and ≥12 drinks/year ("Had at least 12 alcohol drinks/1 year?"). Serum cotinine was dichotomized as ≤10 ng/mL and >10 ng/mL, a cutoff previously used as a marker for both active smoking and high environmental tobacco smoke exposure (Pirkle et al., 1996). Participants were classified as Caucasian, African American, or Other. For 10 of the 89 participants who were missing PIR data, which was used as a continuous measure, we imputed median PIR using values obtained from each survey cycle. Total serum lipids were calculated using the formula: lipids=[total cholesterol (mg/dL) x 2.27] + triglycerides (mg/dL) + 62.3 (CDC, 2006). Lipids, CRP, and GGT were used as continuous measures. Finally, we evaluated medications that can affect hormone homeostasis including antidiabetic and thyroid hormones. Medications used in the past 30 days were identified using the Multum Lexicon Therapeutic Classification Scheme located in the Prescription Medications-Drug Information questionnaire.

5. Statistical analyses

Statistical analyses were performed with SAS® 9.2 (SAS Institute Inc., Cary, North Carolina) without the use of sample weights due to the limited sample size. Similar results were obtained when statistical analyses were repeated with SAS-Callable SUDAAN® 11.0 (Research Triangle Institute, Research Triangle Park, North Carolina) using a four-year sample weight provided by NHANES for the dioxins subsample and nest variables that accounted for the complex survey design. Natural log transformations of exposure variables, CRP, GGT, and lipids were used to approximate a normal distribution, with geometric means presented for descriptive purposes. We used sample weights (wet weight) of PCB, PCDD, and PCDF congeners instead of lipid-standardized measurements for all analyses (Schisterman et al., 2005). We decided a priori to include age, BMI, and lipids as covariates for all final analyses. Differences in demographics, health and lifestyle factors, medication use, and study cycle for POPs and hormones were examined using Student's t-tests for continuous variables or Chi-square tests for dichotomous variables. We used analysis of variance with Tukey post hoc testing to evaluate differences in exposure or outcome measures among race categories. Associations among continuous variables were tested with Pearson's correlation coefficients and partial correlation coefficients. To test for nonlinearity, we evaluated quadratic equations using polynomial regression, although none of the quadratic terms (exposure squared) were significant. Linear regression modeling was used to evaluate potential confounders and effect modifiers. Effect modification was assessed using variables indicating the product of the potential effect modifier with the exposure in hierarchical regression models. When significant interaction was found ($p<.05$), conditional effects were estimated and reported. In order to evaluate confounding, additional covariates were added individually to the adjusted model. Confounding was defined as a change in the exposure beta coefficient of more than 10% after the addition of a potential confounder. We determined GGT to be an important confounder; therefore, associations were estimated with and without additional adjustment for GTT. Results were considered significant at $p<.05$ and borderline significant at $.05<p<.10$.

B. Results

1. Descriptive statistics

Table VIII summarizes continuous characteristics for postmenopausal participants. The mean age was 54.3 years (range 42–60 years) and mean BMI was 29.5 kg/m^2 (range 18.4–46.9 kg/m^2). The mean FSH level was 68.6 and ranged from 27.9 to 196.1. The mean LH level was 37.6 and ranged from 10.2 to 91.0. Mean levels of both hormones were within laboratory reference range; however, one participant had an FSH level notably above the reference range. Table IX summarizes categorical characteristics for postmenopausal participants. About 34% had elevated cotinine levels and medication use varied, with 11.2% using thyroid hormones and 18% using antidiabetic medications.

Table VIII

CONTINUOUS CHARACTERISTICS FOR 89 ELIGIBLE POSTMENOPAUSAL WOMEN, NHANES 1999–2002

Characteristic	n	Mean or geometric mean[a]	95% CI	Minimum or geometric minimum	Maximum or geometric maximum
Ln∑TEQs (pg/g)	66	0.11	0.09–0.13	0.03	2.6
LnPCB 138 (ng/g)	88	0.23	0.19–0.27	0.02	5.6
LnPCB 153 (ng/g)	88	0.33	0.27–0.39	0.02	7.7
LnPCB 180 (ng/g)	89	0.22	0.18–0.26	0.02	4.1
Ln∑PCBs (ng/g)	70	1.2	1.2–1.8	0.3	32.8
Ln∑Non-dioxin-like PCBs (ng/g)	87	1.3	1.1–1.5	0.2	27.3
Ln∑Mono-*ortho* PCBs (ng/g)	89	0.19	0.17–0.23	0.06	5.50
Ln∑Dioxin-like PCBs (ng/g)	70	0.20	0.16–0.24	0.06	5.50
Ln∑Cooke estrogenic PCBs (ng/g)	89	0.39	0.33–0.46	0.04	9.30
Ln∑Wolff estrogenic PCBs (ng/g)	87	0.11	0.09–0.12	0.04	3.10
Ln∑Cooke anti-estrogenic PCBs (ng/g)	70	0.083	0.071–0.097	0.032	1.700
Ln∑Wolff anti-estrogenic PCBs (ng/g)	70	0.28	0.24–0.34	0.08	6.60
FSH (mIU/mL)[b]	89	68.6	62.8–74.4	27.9	196.1
LH (mIU/mL)[c]	89	37.6	34.4–40.9	10.2	91.0
Age (years)	89	54.3	53.3–55.3	42.0	60.0
BMI (kg/m^2)	89	29.5	28.3–30.7	18.4	46.9
LnLipids (mg/dL)	89	675.1	647.3–704.0	458.1	1,499.0
LnCRP (mg/dL)	89	0.32	0.26–0.39	0.04	2.00
Family PIR	89	2.5	2.2–2.8	0.4	5.0
LnGGT (U/L)	89	27.0	23.0–31.7	8.0	1,154.0

[a]Means did not differ by study cycle.
[b]Normal FSH levels for postmenopausal women are 25.8–134.8 mIU/mL.
[c]Normal LH levels for postmenopausal women are 10.0–54.7 mIU/mL.
CI=Confidence interval

TABLE IX

CATEGORICAL CHARACTERISTICS FOR 89 ELIGIBLE POSTMENOPAUSAL WOMEN, NHANES 1999–2002

Characteristic	Percent of total[a]
Age 42–50 years	21.4
51–55 years	32.6
56–60 years	46.1
Alcohol consumption ≥12 drinks/year	52.8
BMI ≥30 kg/m^2 (obese)	46.1
Cotinine >10 ng/mL	33.7
Race/ethnicity	
Caucasian	36.0
African American	24.7
Other	39.3
Antidiabetics	18.0
Thyroid hormones	11.2
Bilateral oophorectomy	11.2
Hysterectomy	24.7

[a]Percent did not differ by study cycle.

For participants with elevated cotinine (>10 ng/mL), 86.7% reported smoking cigarettes every day or some days (data not shown). About 1% of study participants used TZDs, a class of antidiabetic drugs that may inhibit estrogen synthesis (data not shown) (Seto-Young et al., 2011). There were no significant differences between the 1999–2000 and 2001–2002 study cycles for continuous or categorical variables.

2. Bivariate analyses

In unadjusted analyses (data not shown), POP exposures, CRP, and GGT were significantly higher in African Americans than Caucasians (data not shown). Body mass index, CRP, and GGT were also significantly higher in antidiabetic medication users, while mean FSH was significantly lower (data not shown). Polychlorinated biphenyl 180 was significantly lower in participants with high cotinine and CRP was significantly lower in participants who specified an alcohol consumption of ≥12 drinks/year. Table Xa and Xb shows unadjusted Pearson's correlation coefficients among continuous measures. Luteinizing

hormone was significantly and positively related to FSH (r=.77), and was inversely associated with ∑TEQs (r=-.26); somewhat weaker associations were noted with ∑mono-*ortho* PCBs (r=-0.22) and ∑Wolff anti-estrogenic PCBs (r=-.20). Significant negative associations were found for GGT with FSH and LH and significant positive associations were observed for GGT with all POP groupings. Family PIR was positively and significantly associated with FSH but not with LH or POPs. In general, POP groupings were significantly and positively associated with lipids.

3. Pearson's partial correlation coefficients

In analyses adjusted for age, BMI, and lipids (Table XI), LH was significantly and inversely associated with ∑TEQs, while inverse associations of ∑mono-*ortho* PCBs and ∑Cooke anti-estrogenic PCBs with LH were of borderline significance. These associations did not remain significant or borderline significant after further adjustment for GGT. We observed inverse partial Pearson's correlation coefficients for associations between additional anti-estrogenic and/or dioxin-like POP groupings and LH. These associations were slightly over the borderline significant cutoff (*p*-values=.13–.15). No significant or borderline significant associations were found between POPs and FSH.

4. Effect modification

Table XII, Appendix B shows *p*-values for interactions of potential effect modifiers and POPs obtained from hierarchical regression models. There was evidence for interaction between POPs and both thyroid hormone use and CRP for associations between POPs and LH. We noted evidence that BMI modified the effect of ∑Cooke anti-estrogenic PCBs on LH. In addition, there was evidence for interaction by BMI, PIR, race, cotinine, and thyroid hormone use for associations between POPs and FSH. There was evidence that CRP modified the effect of ∑Cooke anti-estrogenic PCBs on FSH.

Table Xa

PEARSON'S CORRELATION COEFFICIENTS FOR 89 ELIGIBLE POSTMENOPAUSAL WOMEN, NHANES 1999–2002

	Ln∑TEQs	Ln∑PCBs	Ln∑NDL	Ln∑MO	Ln∑DL	Ln∑CE	Ln∑WE	Ln∑CA	Ln∑WA
Ln∑TEQs		0.72*	0.70*	0.81*	0.81*	0.68*	0.79*	0.75*	0.79*
Ln∑PCBs	0.72*		0.99*	0.92*	0.92*	0.99*	0.94	0.92*	0.92*
Ln∑NDL	0.70*	0.99*		0.89*	0.89*	0.99*	0.93*	0.91*	0.90*
Ln∑MO	0.81*	0.92*	0.89*		0.99*	0.87*	0.82*	0.94*	0.99*
Ln∑DL	0.81*	0.92*	0.89*	0.99*		0.89*	0.83*	0.93*	0.99*
Ln∑CE	0.68*	0.99*	0.99*	0.87*	0.89*		0.89*	0.89*	0.90*
Ln∑WE	0.79*	0.94*	0.93*	0.82*	0.83*	0.89*		0.85*	0.82*
Ln∑CA	0.75*	0.92*	0.91*	0.94*	0.93*	0.89*	0.85*		0.93*
Ln∑WA	0.79*	0.92*	0.90*	0.99*	0.99*	0.90*	0.82*	0.93*	
FSH	-0.12	0.0046	-0.015	-0.14	-0.079	-0.040	-0.065	-0.004	-0.056
LH	-0.26*	-0.13	-0.11	-0.22*	-0.19	-0.14	-0.13	-0.17	-0.20
Age	0.018	0.0013	0.046	0.065	0.027	0.021	0.028	-0.021	0.057
BMI	0.070	-0.053	-0.066	0.037	0.041	-0.035	-0.050	-0.078	0.012
LnLipids	0.46*	0.24*	0.17	0.29*	0.33*	0.17	0.16	0.33*	0.31*
LnCRP	0.21	0.0086	-0.071	0.080	0.14	-0.062	-0.036	0.041	0.13
PIR	-0.11	-0.034	0.030	-0.079	-0.12	0.077	-0.10	-0.014	-0.094
LnGGT	0.59*	0.41*	0.36*	0.42*	0.45*	0.33*	0.46*	0.41*	0.43*

Table Xb

	FSH	LH	Age	BMI	LnLipids	LnCRP	PIR	LnGGT
Ln∑TEQs	-0.12	-0.26*	0.018	0.070	0.46*	0.21	-0.11	0.59*
Ln∑PCBs	0.0046	-0.13	0.0013	-0.053	0.24*	0.0086	-0.034	0.41*
Ln∑NDL	-0.015	-0.11	0.046	-0.066	0.17	-0.071	0.030	0.36*
Ln∑MO	-0.14	-0.22*	0.065	0.037	0.29*	0.080	-0.079	0.42*
Ln∑DL	-0.079	-0.19	0.027	0.041	0.33*	0.14	-0.12	0.45*
Ln∑CE	-0.040	-0.14	0.021	-0.035	0.17	-0.062	0.077	0.33*
Ln∑WE	-0.065	-0.13	0.028	-0.050	0.16	-0.036	-0.10	0.46*
Ln∑CA	-0.004	-0.17	-0.021	-0.078	0.33*	0.041	-0.014	0.41*
Ln∑WA	-0.056	-0.20	0.057	0.012	0.31*	0.13	-0.094	0.43*
FSH		0.77*	-0.16	-0.38*	-0.022	-0.16	0.23*	-0.22*
LH	0.77*		-0.17	-0.26*	-0.087	-0.15	0.073	-0.24*
Age	-0.16	-0.17		0.0062	-0.10	-0.076	0.032	-0.17
BMI	-0.38*	-0.26*	0.0062		0.026	0.46*	-0.079	0.066
LnLipids	-0.022	-0.087	-0.10	0.026		0.19	-0.097	0.26*
LnCRP	-0.16	-0.15	-0.076	0.46*	0.19		-0.26*	0.26*
PIR	0.23*	0.073	0.032	-0.079	-0.097	-0.26*		-0.33*
LnGGT	-0.22*	-0.24*	-0.17	0.066	0.26*	0.26*	-0.33*	

*p<.05
NDL=non-dioxin-like PCBs
MO=mono-*ortho* PCBs
DL=dioxin-like PCBs
CE=Cooke estrogenic PCBs
WE=Wolff estrogenic PCBs
CA=Cooke anti-estrogenic PCBs
WA=Wolff anti-estrogenic PCBs

TABLE XI

PEARSON'S PARTIAL CORRELATION COEFFICIENTS FOR ASSOCIATIONS OF POPs WITH FSH AND LH AMONG 89 ELIGIBLE POSTMENOPAUSAL WOMEN, NHANES 1999–2002

		FSH (mIU/mL)				LH (mIU/mL)			
POP	**n**	**r[a]**	***p*-value**	**r[b]**	***p*-value**	**r[a]**	***p*-value[c]**	**r[b]**	***p*-value**
Ln∑TEQs (pg/g)	66	-0.11	0.40	0.059	0.65	-0.25	**0.045**	-0.14	0.27
LnPCB 138 (ng/g)	88	-0.026	0.81	0.048	0.67	-0.078	0.48	-0.0089	0.94
LnPCB 153 (ng/g)	88	-0.045	0.68	0.036	0.74	-0.10	0.34	-0.030	0.79
LnPCB 180 (ng/g)	89	-0.018	0.87	0.073	0.51	-0.11	0.30	-0.033	0.76
Ln∑PCBs (ng/g)	70	-0.017	0.89	0.097	0.44	-0.14	0.27	-0.048	0.70
Ln∑Non-dioxin-like PCBs (ng/g)	87	-0.031	0.78	0.061	0.59	-0.11	0.30	-0.032	0.77
Ln∑Mono-*ortho* PCBs (ng/g)	89	-0.12	0.27	-0.024	0.83	-0.19	**0.07**	-0.11	0.33
Ln∑Dioxin-like PCBs (ng/g)	70	-0.062	0.62	0.056	0.65	-0.18	0.15	-0.084	0.50
Ln∑Cooke estrogenic PCBs (ng/g)	89	-0.060	0.65	0.031	0.78	-0.14	0.20	-0.065	0.56
Ln∑Wolff estrogenic PCBs (ng/g)	87	-0.084	0.45	0.032	0.78	-0.13	0.23	-0.027	0.81
Ln∑Cooke anti-estrogenic PCBs (ng/g)	70	-0.046	0.71	0.062	0.62	-0.20	**0.098**	-0.12	0.32
Ln∑Wolff anti-estrogenic PCBs (ng/g)	70	-0.039	0.75	0.078	0.53	-0.19	0.13	-0.10	0.44

[a]Models adjusted for age, BMI, and lnLipids.

[b]Models adjusted for age, BMI, lnLipids, and lnGGT.

[c]*P*-values in bold are statistically significant or borderline significant at $p<0.10$.

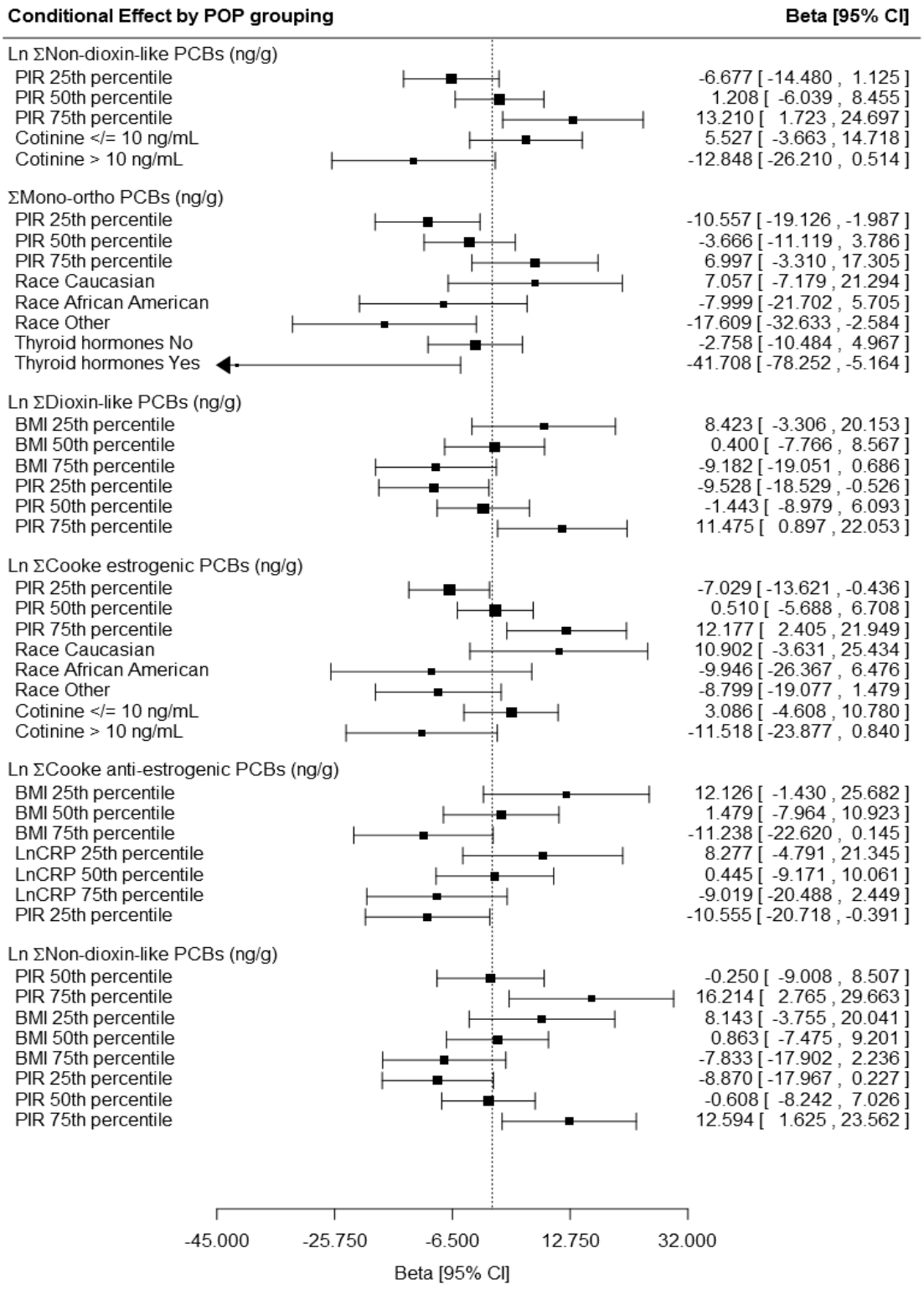
r=Correlation coefficient

To help understand the pattern of interaction, we displayed dichotomized estimates and estimates centered at the 25th, 50th, and 75th percentiles of continuous potential effect modifiers (Table XII, Appendix B). For associations of POP groupings with LH, we found significant inverse associations for thyroid hormone users and CRP centered at the 75th percentile. For associations of POP groupings with FSH, we found significant inverse associations of four participants who were in the Other race category (not Caucasian or African American), thyroid hormone users, elevated cotinine (>10 ng/mL), or BMI centered at the 75th percentile. In addition, effects depended on PIR, with inverse associations of POPs with FSH in participants with low PIR and positive associations in participants with high PIR. Significant interactions with thyroid hormones occurred in models both with or without additional adjustment for GGT, and significant interactions with cotinine were generally attenuated after further adjustment for GGT. When POPs were evaluated according to classification schemes, associations of anti-estrogenic and/or dioxin-like POP groupings with FSH depended on BMI, with stronger effects in obese participants. Selected associations of POPs and hormones by potential effect modifiers are presented in Figures 2 and 3.

5. Sensitivity analyses

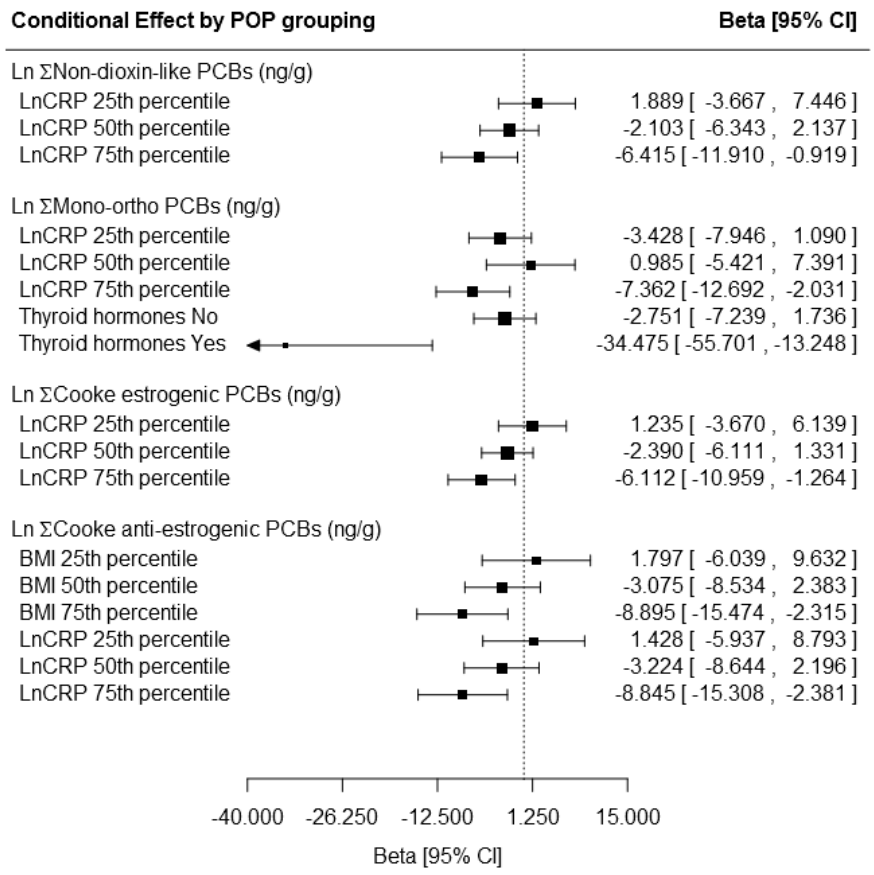
Lipid standardization of POPs, or the division of serum POP concentrations by lipids, may introduce bias (Schisterman et al., 2005); therefore, we used wet weight POP measurements controlling for serum lipids. Results were similar when we repeated the analysis using lipid-standardized exposure measurements unadjusted for lipids. The majority of participants with the absence of menstrual periods for 12 months or more specified the reason for not having regular periods as "going/gone through menopause." Only three postmenopausal women specified "medical conditions/treatments" as the reason for not having regular menstrual periods (two of the three were 60 years of age). Exclusion of these participants in a sensitivity analysis yielded similar results, except for the borderline significant correlation between ∑Cooke anti-estrogenic PCBs and LH, which became significant. Repeating the analysis with exclusion of an FSH level notably outside of the laboratory range produced results that were unchanged. Because body burdens of dioxins and PCBs have declined over time and individual results below the LOD differed by study cycle, we conditioned estimates on study cycle. Overall, we found similar results for the 1999–2000 study cycle, although associations were not statistically significant for the 2001–2002 study cycle. Compared with natural menopause, oophorectomy in postmenopausal women was shown to lower androgen levels (Laughlin et al., 2000; Labrie et al., 2011); therefore, we repeated the current analysis excluding postmenopausal participants with bilateral oophorectomy. This exclusion yielded similar results. Finally, one participant reported the use of TZDs, which are a class of insulin-sensitizing agents used to treat diabetes and may affect estrogen metabolism as a result of inhibition of aromatase activity (Seto-Young et al., 2011). When we repeated the analysis excluding this participant, similar results were observed. Statistical analyses were repeated with SAS-Callable SUDAAN 11.0 (Research Triangle Institute, Research Triangle Park, North

Carolina) using a four-year sample weight provided by NHANES for the dioxins subsample and nest variables that accounted for the complex survey design. Weighting the analysis yielded similar results with somewhat attenuated significance.



Box size indicates the precision of the estimate; larger boxes signify less variance.
A confidence interval (CI) that extends beyond the plot domain is indicated by an arrow.

Figure 2. Selected associations of POPs with FSH by potential effect modifiers for 89 eligible postmenopausal women, NHANES 1999–2004.



Box size indicates the precision of the estimate; larger boxes signify less variance.
A confidence interval (CI) that extends beyond the plot domain is indicated by an arrow.

Figure 3. Selected associations of POPs with LH by potential effect modifiers for 89 eligible postmenopausal women, NHANES 1999–2004.

C. Discussion

1. Follicle-stimulating hormone and luteinizing hormone

In this investigation using 1999–2002 NHANES data, we found an inverse association of exposure to ∑anti-estrogenic and/or dioxin-like POP groupings with LH, but not FSH, in postmenopausal women not taking glucocorticoids or sex hormones. Further adjustment for GGT attenuated these associations. Inverse associations of POPs with LH were stronger in participants using thyroid hormones or with elevated CRP, while inverse associations of POPs with FSH were stronger and significant in participants who were in the Other race category (not Caucasian or African American), obese, taking thyroid hormones, or who had elevated cotinine. Additionally, the associations between POPs and FSH depended on PIR, with negative associations in participants with low PIR and positive associations in participants with high PIR. Associations of anti-estrogenic and/or dioxin-like POP groupings with FSH depended on BMI, with stronger inverse effects in obese participants.

In a previous investigation of postmenopausal women with occupational exposures, PCBs were inversely associated with FSH and SHBG (Persky et al., 2011). In general, research has shown an inverse association between PCB exposure and SHBG-related measures, suggesting that PCBs may affect the binding of estrogen. Estrogenic environmental chemicals acting as estrogen may also increase SHBG. Our results, however, are not consistent with epidemiologic studies that have explored possible associations of PCBs and TCDD with FSH and LH in men. In several studies representing a range of exposure levels, POPs were not generally associated with FSH or LH. In healthy men from the West Indies, PCB 153 was positively associated with androstenedione and estrone levels, but was not related to FSH or LH (Emeville et al., 2013). In men from a US fertility clinic, PCB 118 was inversely associated with SHBG but was not related to FSH or LH (Ferguson et al., 2012). In Inuits and several European cohorts, positive associations of PCB 153 with SHBG and LH were observed in some but not all groups (Giwercman et al., 2006). In a study of young Swedish men from the general population, PCB 153 was inversely associated with the testosterone-to-SHBG ratio but was not related to FSH or LH (Richthoff et al., 2003). Polychlorinated biphenyl 153 was not associated with FSH and LH in men exposed to high levels of POPs through fish consumption (Hagmar et al., 2001). In occupationally exposed men, TCDD was significantly and positively associated with FSH and LH (Sweeney et al., 1997). In an investigation of male Great Lakes fish consumers, PCBs were not related to FSH or LH; however, significant inverse associations of PCBs with SHBG-bound testosterone were noted (Persky et al., 2001). In addition, occupational exposures to PCBs were not associated with FSH or LH in men (Persky et al., 2012). A lack of association between POPs and the hormones FSH and LH in some studies could be indicative of a threshold of bioavailable estrogen necessary for impacts on circulating hormones. Further, the effect of mixtures of PCBs, PCDDs, and PCDFs might be additive or antagonistic, depending on the population, dose, and endpoints.

Surprisingly, we found that anti-estrogenic and/or dioxin-like POP groupings were negatively associated with LH. Inverse relationship of POPs with LH may be a marker for an exogenous source of bioavailable estrogen. Because the 1999–2002 NHANES data sets do not provide measurements of other estrogen-related hormones for women, it is difficult to postulate potential mechanisms. Dioxins and related compounds that bind to the AhR generally elicit anti-estrogenic responses (Safe et al., 1998). The findings of associations between anti-estrogenic and/or dioxin-like POP groupings with LH appear counterintuitive, given that LH levels were decreased, which implies an estrogenic effect. However, our findings may be consistent with previous research suggesting cross-talk between the estrogen receptor and the AhR signaling pathways (Cooke et al., 2008; Swedenborg and Pongratz, 2010). Further, dioxin-like and potentially anti-estrogenic PCBs have been associated with increased gene expression of estrogen receptor beta and CYP19 coding for aromatase, an enzyme involved in estrogen synthesis (Warner et al., 2012).

The literature has shown, among multiple factors that contribute to LH secretion, that estrogen plays an important role by exerting feedback to the pituitary in the normal functioning of the HPG axis (Clarke, 2002; Christian et al., 2005); however, the mechanism by which estrogen controls these events has not been delineated. Because control of FSH secretion is more complex than LH and includes stimulus by inhibins and activins, FSH is not considered to be a good marker for estrogen-negative feedback control of gonadotropin secretion (Weiss et al., 2004). New research challenges this view by demonstrating that the effect of estrogen on FSH responsiveness is greater than on LH (Cosma et al., 2008; Shaw et al., 2010).

2. <u>Effect modification</u>

Inverse associations of anti-estrogenic and/or dioxin-like PCB groupings with FSH in the present study were significant in obese postmenopausal participants. There is evidence that body composition may play an important role in steroid hormones and SHBG concentrations. In general, the aromatization of androstenedione to estrone in adipose tissue correlates positively with weight (Bulun et al., 1994). Inverse correlations have also been reported between BMI and SHBG for postmenopausal women, resulting in increased bioavailable estradiol (Cauley et al., 1989).

Findings of significant inverse associations of POP groupings with FSH and LH among postmenopausal women taking thyroid hormone medications might be expected given that the metabolism of both androgens and estrogens is altered in hypothyroidism. Androgen production is decreased, and the metabolism of testosterone is shifted toward etiocholanolone rather than androsterone. “With respect to estradiol and estrone, hypothyroidism favors metabolism of these steroids via 16α-hydroxylation over 2-oxygenation, resulting in increased formation of estriol at the expense of 2-hydroxyestrone and its derivative, 2-methohyestrone” (Doufas and Mastorakos, 2000). The SHBG in plasma is decreased, resulting in a decline of

plasma concentrations for both testosterone and estradiol, though the unbound fractions are increased (Brenta et al., 1999). We did not find evidence of effect modification by antidiabetics in the current study, even though antidiabetic medication use in the past 30 days was significantly associated with BMI with and without adjustment for age.

Inverse associations of POPs with LH were significant in postmenopausal women with high CRP levels. C-reactive protein is a general marker of systematic inflammation. Several studies have noted associations of CRP with factors relating to estrogen status, including higher levels of estradiol and lower levels of SHBG (Stork et al., 2008; Maggio et al., 2011), as well as stimulation of aromatase (Zhao et al., 1996). Some investigators have suggested that PCBs may act through increased inflammatory responses (Hennig et al., 2002). In the current study, CRP was associated with antidiabetic medications, BMI, and triglycerides after adjusting for potential confounders (data not shown).

Much less is known about the association of modifiable factors such as cigarette smoking with hormones. In some investigations of men, current smokers had higher circulating concentrations of SHBG (English et al., 2001; Allen et al., 2002; Muller et al., 2003; Svartberg et al., 2003; Svartberg and Jorde, 2007; Shiels et al., 2009) but not after adjustment for testosterone or estradiol (Shiels et al., 2009). Findings of significant inverse associations of estrogenic and/or non-dioxin-like PCB groupings with FSH among postmenopausal women with elevated cotinine are unexpected given the known anti-estrogenic effect of cigarette smoke. Previous investigations have found smokers to have lower levels of PCDFs and dioxin-like PCBs in serum (Flesch-Janys et al., 1996; Chen et al., 2005; Ferriby et al., 2007; Jain et al., 2011) and milk (Takekuma et al., 2004; Hedley et al., 2006; Uehara et al., 2007) when compared with nonsmokers. It was suggested that increased levels of smoking resulted in lower concentrations of these chemicals by enhancing their elimination and, in turn, reducing body burdens (Takekuma et al., 2004; Chen et al., 2005). In the current study, we generally found higher concentrations of POPs in participants with elevated cotinine; however, only PCB 180 was statistically significant.

Positive associations of POPs with FSH were observed in postmenopausal women with high PIR levels, while negative associations were noted in participants with low PIR levels. Further, negative associations were noted for ∑mono-*ortho* PCBs and ∑Cooke estrogenic PCBs with FSH in participants who were in the Other race category. Adverse health outcomes such as asthma, cancer, and diabetes are related to low socioeconomic status (SES). The literature has hypothesized that disease disparities may result from increased exposure to environmental contaminants. Different contaminants accumulate with different patterns according to SES. For example, previous investigations have noted social and racial disparities in PCB exposure. In the current investigation, African Americans had significantly higher POP exposures than Caucasians. Conversely,

high SES individuals generally have a different exposure profile, reflecting variations in fish and shellfish consumption and sunscreen use (Tyrrell et al., 2013).

A liver enzyme associated with oxidative stress, GGT may potentially be an important confounder in the association of POPs with hormones. For associations of POPs with FSH and LH, significant interactions with thyroid hormone use occurred in models with or without additional adjustment for GGT. For associations of POPs with FSH, significant interactions with cotinine were generally attenuated after further adjustment for GGT. Our findings of significant positive correlations between POP body burdens and GGT and inverse correlations of hormones with GGT are potentially of biologic significance. Previous investigations have suggested a relationship between GGT with POPs (Lee and Jacobs, 2006), known markers of inflammation (Lee and Jacobs, 2005), and type 2 diabetes (Lee et al., 2003a; Lee et al., 2003b). To our knowledge, no investigations have examined the effect of GGT on FSH or LH. Estimates further adjusted for GGT should be interpreted with caution given the small sample size and its association with lipids, although there was no evidence of multicollinearity upon examination of variance inflation factors.

3. Limitations and strengths

The present investigation has a number of limitations. The cross-sectional design of the study does not allow us to establish a temporal relationship of POP exposures with changes in gonadotropin levels. Findings may be affected by lack of power to detect associations between POPs and hormones due to limited sample size. The associations conditioned on the 2001–2002 study cycle were generally not statistically significant and may be attributed to study cycle differences in congener measurements below the LOD in the first study cycle. Body burdens of POPs did not differ by study cycle. In addition, because factors including BMI were found to modify the association of POP exposures with hormones, adjusting for these variables in multiple linear regression analyses may not be appropriate. We adjusted for age, BMI, and lipids with and without additional adjustment for GGT, but there are other important potential confounders for which we have not controlled. Additional congener measurements for complete congener groupings might have helped to better elucidate mechanisms related to associations between PCB groupings and hormones. Estrogenic and anti-estrogenic classes of PCB congeners can express diverse and sometimes conflicting effects (Warner et al., 2012). Finally, the findings might be due to chance, as multiple comparisons were made in the statistical analysis.

Despite the limitations, our investigation has a number of strengths. Our investigation adds to epidemiologic literature and increases the confidence that associations we have reported are not the product of chance due to some similar trends and consistency with a previous investigation. To our knowledge, only one other study has evaluated the association of POPs on hormones in postmenopausal women. We also investigated the associations using continuous data and centering

potential effect modifiers to ensure we did not further limit the power of our analysis. Finally, multiple exposures groupings and confounders were evaluated.

D. Conclusion

In this investigation, we found significant and borderline significant inverse associations of anti-estrogenic and/or dioxin-like PCB groupings with LH in postmenopausal women. Although not statistically significant, PCBs were also inversely associated with FSH. Overall, inverse associations were noted between POPs and FSH for participants who were Other race, obese, taking thyroid hormones, had elevated cotinine, and had high PIR levels. In addition, there was evidence of effect modification, with several factors perhaps reflecting variations in estrogen profile. In future studies, it may be important to include potential effect modifiers when evaluating associations of PCBs with hormones.

V. HORMONE DISRUPTION BY POLYCHLORINATED BIPHENYLS IN POSTMENOPAUSAL WOMEN FROM A COHORT OF GREAT LAKES SPORT FISH CONSUMERS

Persistent organic pollutants, such as PCBs, are noted endocrine disruptors. Polychlorinated biphenyls are a class of heat-resistant, oily liquids that were manufactured in the United States from the 1920s until the 1970s and were used as insulating fluids in capacitors and transformers (ATSDR, 2000). These chemicals are resistant to abiotic and biotic degradation and biomagnify in the food chain (Safe, 1994; Van den Berg et al., 1998). Because of their low water solubility and high lipid solubility, PCBs deposit in adipose tissue and are persistent in humans. Body burdens of these chemicals have declined over time (Aylward et al., 2002; Turyk et al., 2012); however, continued exposure is principally associated with contamination of food items (van Larebeke et al., 2001). Previous studies of this cohort of Great Lakes fish consumers have shown that PCBs were significantly correlated with frequency of consuming sport fish and Great Lakes sport-caught fish (Hanrahan et al., 1999).

Some PCB congeners are similar to estradiol in terms of chemical structure and mimic its effects (Cooke et al., 2001). Low chlorinated PCB congeners are suggested to have estrogenic activities. Polychlorinated biphenyl congeners that are *ortho*-substituted do not bind to the AhR, whereas the non- or mono-*ortho* coplanar PCBs have a high affinity for the AhR (Poland et al., 1985; Safe et al., 1985; Van den Berg et al., 1998). The AhR is a member of transcriptional regulators that may control a variety of physiological events including hormone receptor function (Pocar et al., 2005). In addition, PCBs were suggested to up-regulate the genes coding for receptors and enzymes involved in steroid metabolism, including estrogen receptor beta and CYP19) which encodes aromatase, the enzyme responsible for converting androgen precursors to estrogen (Warner et al., 2012). A recent investigation has suggested that dioxins and dioxin-like POPs up-regulate genes related to adiposity, insulin resistance, and inflammation (Kim et al., 2012). It is not currently apparent if these mechanisms are relevant for POP effects on hormones or binding proteins such as SHBG. Conversely, some PCBs are similar to estradiol in terms of chemical structure and, thus, a potential mechanism for estrogenic PCBs includes the capacity to bind and activate estrogen receptors (Cooke et al., 2001) or bind to SHBG (Hodgert et al., 2000). In addition, some PCBs and their metabolites inhibit estrogen sulfotransferase, an enzyme that inhibits estrogen metabolism (Kester et al., 2000; Kester et al., 2002).

Polychlorinated biphenyls have been associated with a multitude of health effects in animal experiments and epidemiologic investigations, including disruption of steroid hormone homeostasis. Steroid hormones are controlled by the HPG axis. Reduction of circulating estradiol after menopause is compensated for by release of the gonadotropins FSH and LH from the pituitary. In cycling women, the increase in FSH and LH in turn stimulates the ovaries to produce more estradiol, whereas after menopause, circulating levels of estrogen are derived from aromatization of androgens in adipose tissue.

Estradiol is the most important inhibitor of gonadotropin secretion; however, estrone becomes the predominant estrogen after menopause.

Overweight women convert more androgens to estrogens than women of normal weight due to increased aromatase activity. The bioavailability of estrogen in circulation is affected by proteins known as SHBG. Estrogen is transported to peripheral tissues primarily bound to SHBG as a complex known as SHBG-E2, but estradiol can also be loosely bound to albumin. Hepatic production of SHBG is affected by the estrogen-androgen balance, with estrogens stimulating and androgens suppressing their synthesis (Bulun et al., 2008). Other health-related factors affecting SHBG levels, such as hypothyroidism, obesity, and inflammation, may influence the bioavailability of estradiol.

Several in vitro studies and investigations in male and female animals have indicated an association between PCBs and alterations in steroid and gonadotropin hormone levels (Desaulniers et al., 1999; Wade et al., 2002; Oskam et al., 2005; Uslu et al., 2013). Polychlorinated biphenyls were found to act directly on gonadotropin-releasing hormone gene expression in hypothalamic GT1-7 cells (Gore et al., 2002). To our knowledge, there is only one study of postmenopausal women that has indicated an association between PCBs and alterations in hormone levels, specifically inverse relationships with FSH and SHBG (Persky et al., 2011). It is well established that men and women differ in hormonal profile. Epidemiologic studies in males suggested an association between PCBs and altered steroid hormones (Richthoff et al., 2003; Turyk et al., 2006; Goncharov et al., 2009; Dhooge et al., 2011; Persky et al., 2011; Ferguson et al., 2012) and SHBG levels (Giwercman et al., 2006; Haugen et al., 2011).

Previous investigations of men from the Great Lakes fish consumption cohort have shown that PCBs were inversely related with SHBG-bound testosterone, but not estrone sulfate, FSH, or SHBG (Persky et al., 2001). In a subgroup of men from the same cohort, models that accounted for both dioxin-like chemicals and PCBs predicted SHBG-bound testosterone (Turyk et al., 2006). In the current study, we extend our analysis to examine the effects of exposure to PCBs on steroid and gonadotropin hormones and binding proteins in a subgroup of 83 postmenopausal women from the GLFCS. This report is focused on the relationships of hormones with several individual PCB congeners, ∑PCBs, and PCB congeners grouped into classes with similar structure or mode of action. In addition, we examined the hypothesis that associations of PCBs with hormones are modified by estradiol and other factors that may potentially increase endogenous estrogens such as BMI.

A. Methods

1. Participants

The protocol for this investigation was reviewed and approved by the University of Wisconsin-Madison Medical School Human Subjects Committee and the University of Illinois at Chicago Human Subjects Review Board. All subjects gave written informed consent prior to the initiation of the study. The GLFCS was organized in the early 1990s and was originally designed to focus on Great Lakes sport fish consumption and reproductive outcomes (Anderson et al., 1996; Hanrahan et al., 1999).

> "Men and women who held 1992 charter boat captain registrations in Illinois, Indiana, Michigan, Ohio or Wisconsin were identified as individuals who were likely to be habitual consumers of Great Lakes sportfish. All charter boat captains were contacted by telephone and invited to participate in this study. Spouses were eligible for inclusion if the couples had at least one child that was born in 1970 or later. The referent group of infrequent consumers of Great Lake fish was recruited during 1994–1995 and matched to charter boat captains by city or town of residence and telephone exchange. Referents were eligible to participate if they had not eaten any Great Lakes sportfish during the previous 12 months and had not eaten more than six sportfish meals in any year since 1970" (Knobeloch et al., 2009).

This cross-sectional study used follow-up survey data that were collected on subgroups of the original GLFCS cohort in 2001–2003 and 2004–2005. Participants provided a blood sample in 2001–2005 for hormone and PCB measurements.

Any associations between PCBs and steroid hormones may be more clearly observed among postmenopausal women who are no longer cycling and are not current users of sex hormones or glucocorticoids. Menopause is defined as one year after the permanent cessation of menstrual periods, which women experience at the average age of 51 years. Menopause can occur naturally or be induced through a medical intervention such as bilateral oophorectomy. Bilateral or unilateral oophorectomy was not specified in the current study, although exclusion of participants with oophorectomy was conducted in a sensitivity analysis. Normal FSH levels for premenopausal women are 4.7–21.5 mIU/mL, while normal FSH levels for postmenopausal women are 25.8–134.8 mIU/mL (Lobo, 2007). The definition of menopause used in the current report was based on a previous investigation (Kalkwarf et al., 2003). Standards were applied consecutively so that the rules were applied only to women not already in a previous category. The resulting inclusion categories for postmenopausal women were:

1. Any age and last period ≥12 months without hysterectomy or with hysterectomy and bilateral oophorectomy
2. ≥60 years of age

3. 56–59 years of age and last period ≥12 months with hysterectomy and without bilateral oophorectomy, and FSH ≥25.8 mIU/mL
4. <56 years of age and last period ≥12 months with hysterectomy and without bilateral oophorectomy, and FSH ≥50 mIU/mL

In the present report, we focused on the PCB measurements of 156 postmenopausal women. Participants who were <40 years of age were excluded because we assumed they experienced early menopause (*n*=1), and we excluded participants <60 years of age with FSH <25.8 mIU/mL because they were potentially pre- or peri-menopausal (*n*=45). Participants were also excluded if they did not have hormone or lipid measures (*n*=3). Complete data for analysis of the associations of PCBs with FSH and estradiol were available for a total of 77 participants after excluding participants who reported taking glucocorticoids (*n*=2) or who specified taking sex hormones (*n*=28). Sex hormone-binding globulin and SHBG-E2 measurements were available for 68 participants.

2. Hormones, binding proteins, and other physiological measurements

Measured hormone levels include estradiol, FSH, SHBG, and SHBG-E2. Hormone assays and binding studies were conducted in the Immunoassay Core Facility of the Robert H. Lurie Comprehensive Cancer Center of Northwestern University. Serum specimens were analyzed by laboratory technicians who were blind to the exposure status of the participants. Estradiol was measured by Ultra-sensitive Estradiol Assay (Diagnostic Systems Laboratories, Inc., Webster, Texas). This assay is a double antibody radioimmunoassay with an antiserum highly specific for estradiol; only estrone (2.4%), estriol (0.64%), and estradiol 3-glucuronide (2.6%) compete significantly for estradiol. The intra- and interassay CVs for serum estradiol were 7.5% and 18.7%, respectively. Follicle-stimulating hormone was measured by a coated tube radioimmunoassay (Diagnostic Systems Laboratories, Inc., Webster, Texas). This assay allowed FSH to be captured between monoclonal anti-FSH antibodies immobilized on the inside surface of the polystyrene tube and the ^{125}I-labeled polyclonal anti-FSH tracer. The sensitivity was approximately 0.1 mIU/mL. Results were expressed in mIU/mL in terms of the World Health Organization Second International Reference Preparation of FSH for bioassay, number 78/549. The intra- and interassay CVs for serum FSH were 6.1% and 12.6%, respectively. Sex hormone-binding globulin was measured by radioimmunoassay kits (Diagnostic Systems Laboratories, Inc., Webster, Texas). This competitive immunoassay had a sensitivity of 5 nmol/L. There is no cross-reactivity with other glycoproteins that may be encountered in serum. The intra- and interassay CVs for serum SHBG were 6.6% and 15.7%, respectively. Sex hormone-binding globulin-bound estradiol was measured as described previously (Bonfrer et al., 1989). Briefly, 0.2 mL of serum diluted 1:8 with buffer was equilibrated with ^{3}H-testosterone overnight at 4°C. A 0.10 mL suspension of a Concanavalin A-sepharose (Con-A-sepharose) conjugate was added to the serum. Sex hormone-binding globulin was allowed

to bind to the Con-A-sepharose during a 30-minute incubation period at room temperature. Estradiol in the serum maintains its equilibrium concentration with SHBG in the presence of endogenous factors such as other estrogens, androgens, and free fatty acids (Bonfrer et al., 1989; Street et al., 1989). Separation of unbound ^{3}H-estradiol from that bound to the Con-A-sepharose was achieved by centrifugation at 0°C in order to minimize dissociation of bound testosterone. The intra- and interassay CVs for SHBG-E2 were 5.1% and 5.4%, respectively. For two of the 68 participants who had SHBG measurements equal to zero, we imputed half of the lowest SHBG value. Total cholesterol and triglycerides were measured by Quest Diagnostics (Auburn Hills, MI, and Wood Dale, Illinois) in samples collected in 2004–2005 and by Meriter Laboratories (Madison, Wisconsin) in samples collected in 2001–2003.

3. Polychlorinated biphenyl measurements

Participants were instructed to abstain from eating fish for 72 hours before a non-fasting blood sample was drawn. Blood for the analyses of individual PCB congeners was collected in red-top Vacutainer tubes, allowed to clot for 20 minutes, centrifuged for 15 minutes, transferred to solvent-rinsed glass vials, and stored at -20°C until analysis. Serum for exposure measurements was extracted with hexane\ethyl ether, with clean-up and fractionation using Florisil, silica-gel, and concentrated sulfuric acid as previously described (Anderson et al., 2008). Polychlorinated biphenyl congeners were analyzed using high-resolution capillary column gas chromatography equipped with an electron capture detector (Burse et al., 1990). Quality control was monitored by the use of method blanks, spiked bovine serum samples, duplicates of bovine serum spikes or sample duplicates, surrogate spikes, and confirmation of the analytes by second column or gas chromatography-mass spectrometry, as appropriate. The various congener groupings used in the current report are presented in Table XIII. Congener-specific values for PCB congeners were summed to yield ∑PCBs. Polychlorinated biphenyl congeners were grouped according to structure including ∑non-dioxin-like PCBs and ∑mono-*ortho* PCBs. We dichotomized ∑mono-*ortho* PCBs at the LOD because of its bimodal distribution. Polychlorinated biphenyl congeners were also grouped according to mechanism of action, including estrogenic and anti-estrogenic activity (Wolff et al., 1997; Cooke et al., 2001). Anti-estrogenic PCB congeners based on Cooke's criteria were lacking; therefore, ∑Cooke anti-estrogenic PCBs are omitted from this report. We also selected three PCB congeners (PCB 138, PCB 153, and PCB 180) for the analysis because they are often the most prevalent congeners found in serum (CDC, 2009). Because of co-elution, PCB 163 was not separated from PCB 138 (PCB 163/138) and PCB 153 was not separated from PCB 132 or 105 (PCB 132/153/105). Further, for PCB 132/153/105, dioxin-like PCB 105 was not separated from non-dioxin-like PCB 153 (Table XIII). According to the National Report on Human Exposure to Environmental Chemicals, however, when summed PCB 105 only contributes 6.6% to the 153/105 total (Lambertino et al., 2011). For congeners with results below the LOD, the measurement was imputed as the LOD for that specific congener divided by two. Measurements

below the LOD were also imputed as the LOD for that specific congener divided by the square root of two in a sensitivity analysis.

4. Covariates

Follow-up surveys completed in 2001–2003 and 2004–2005 assessed information on demographics, height, weight, cigarette smoking, alcohol consumption, reproductive health, and medication use. Potential confounders and effect modifiers evaluated in this study included age, alcohol consumption, BMI, CRP, GGT, lipids, cigarette smoking, race, and antidiabetic or thyroid hormone medication use. Alcohol consumption was dichotomized as <12 drinks/year and ≥12 drinks/year. Cigarette smoking was dichotomized as nonsmokers and current smokers. Body mass index was calculated using weight and height measurements. Total serum lipids were calculated using the formula: lipids=(total cholesterol (mg/dL) x 2.27) + triglycerides (mg/dL) + 62.3. Age, BMI, CRP, GGT, and lipids were analyzed as continuous variables.

TABLE XIII

CONGENER GROUPINGS, GLFCS

Grouping	Congeners[a]
∑PCBs[b]	**66, 74, 99, 118, 128, 146, 167, 172, 177, 178, 180, 183, 193, 194, 201, 206, 163/138, 170/190, 203/196, 202/171, 208/195, 187/182, 132/153/105**
∑Non-dioxin-like PCBs[b]	**66, 74, 99, 128, 146, 172, 177, 178, 180, 183, 194, 206, 163/138, 170/190, 203/196, 208/195, 187/182, 132/153/105[d]**
∑Mono-*ortho* PCBs	105, 114, **118**, 123, 156, 157, **167**, 189
∑Cooke estrogenic PCBs	1, 3, 4, 8, 15, 18, 21, 31, 44, 47, 48, 49, 52, 54, 61, 70, 75,77, 80, 95, **99**, 101, 104, 110, 126, 136, **132/153/105[c]**, 155, 184, 188
∑Wolff estrogenic PCBs	101, 174, **177**, **187/182**, **201**
∑Wolff anti-estrogenic PCBs	**66**, **74**, 77, **118**, 126, 155, 156, 169

[a]Congeners in bold were included in the grouping.
[b]Only measured congeners in the grouping are shown.
[c]Non-dioxin-like PCB 153 co-eluted with dioxin-like PCB 105.

CRP and GGT were measured in a subgroup of participants in 2004–2005 (*n*=63). We also evaluated estradiol as a potential effect modifier of the effect PCBs on FSH, SHBG, and SHBG-E2. We decided a priori to include age, BMI, and lipids as covariates for all final analyses.

5. Statistical analyses

Statistical analyses were performed with SAS® 9.2 (SAS Institute Inc., Cary, North Carolina). Natural log transformations of exposure variables, estradiol, CRP, and GGT were used to approximate a normal distribution, with geometric means presented for descriptive purposes. For all analyses, we used sample weights (wet weight) of PCB congeners rather than lipid-standardized measurements (Schisterman et al., 2005). Differences in demographics, health and lifestyle factors, and medication use for PCBs and hormones were examined using Student's t-tests for continuous variables. Chi-square tests or Fisher's exact tests were used for dichotomous variables, as appropriate. Associations among continuous variables were tested with Pearson's correlation coefficients and partial correlation coefficients. We evaluated quadratic equations using polynomial regression to test for nonlinearity; however, no quadratic terms (exposure squared) were significant. Linear regression modeling was used to evaluate potential confounders and effect modifiers. Effect modification was evaluated using variables indicating the product of the potential effect modifier with the exposure in hierarchical regression models. When significant or borderline significant interaction was found, conditional effects were estimated and reported. To assess confounding, additional covariates were added individually to the adjusted model. Confounding was defined as a change in the exposure beta coefficient of more than 10% after the addition of a potential confounder. Results were considered significant at $p<.05$ or borderline significant at $0.5< p<.10$.

B. Results

1. Descriptive statistics

Table XIV summarizes continuous characteristics for postmenopausal participants. The mean age was 59.3 years (range 46–79 years) and mean BMI was 27.9 kg/m^2 (range 17.8–49.6 kg/m^2). The mean FSH level was 64.2 and ranged from 15.6 to 177.2. The estradiol geometric mean was 14.5 and ranged from 4.9 to 51.4. The mean SHBG level was 228.9 and ranged from 6.9 to 630.0. Mean or geometric mean levels of FSH, estradiol, and SHBG were within laboratory reference range; however, one participant had an FSH level notably above the reference range (Table XIV).

Table XV summarizes categorical characteristics for postmenopausal participants. Ninety-seven percent of the participants were Caucasian. Approximately 4% were cigarette smokers and medication use varied, with 19.5% using thyroid hormones and 6.5% using antidiabetic medications. Twelve out of 77 participants or 15.6% were referents who reported no

consumption of Great Lakes sport fish during the previous 12 months and had not eaten more than six sport fish meals in any year since 1970. Finally, 60% of participants had ∑mono-*ortho* PCB levels below the LOD (Table XV).

TABLE XIV

CONTINUOUS CHARACTERISTICS FOR 77 ELIGIBLE POSTMENOPAUSAL WOMEN, GLFCS

Characteristic	n	Mean or geometric mean[a]	95% CI	Minimum or geometric minimum	Maximum or geometric maximum
LnPCB 163/138 (ng/g)	77	0.36	0.30–0.42	0.08	2.10
LnPCB 132/153/105 (ng/g)	77	0.36	0.31–0.41	0.08	1.70
LnPCB 180 (ng/g)	77	0.32	0.28–0.37	0.05	1.10
Ln∑PCBs (ng/g)	77	2.4	2.2–2.7	1.2	6.9
Ln∑Non-dioxin-like PCBs (ng/g)	77	2.1	1.8–2.3	0.9	6.5
Ln∑Cooke estrogenic PCBs (ng/g)	77	0.43	0.37–0.49	0.13	1.90
Ln∑Wolff estrogenic PCBs (ng/g)	77	0.24	0.20–0.27	0.12	0.83
Ln∑Wolff anti-estrogenic PCBs (ng/g)	77	0.27	0.24–0.30	0.20	1.00
FSH (mIU/mL)[a]	77	64.2	58.2–70.1	15.6	177.2
LnEstradiol (pg/mL)[b]	77	14.5	13.3–15.9	4.9	51.4
SHBG (nmol/L)[c]	68	228.9	196.8–261.1	6.9	630.0
SHBG-E2 (percent)	68	21.3	19.1–23.5	5.4	42.2
FSH:E2	77	4.8	4.2–5.4	0.57	13.90
Age (years)	77	59.3	58.0–60.6	46.0	79.0
BMI (kg/m^2)	77	27.9	26.6–29.2	17.8	49.6
Lipids (mg/dL)	77	707.2	681.4–733.1	463.3	955.5
LnCRP (mg/dL)	63	0.23	0.19–0.29	0.03	1.10
LnGGT (U/L)	63	17.9	15.4–20.8	7.0	358.0

[a]Normal FSH levels for postmenopausal women are 25.8–134.8 mIU/mL.

[b]Normal estradiol levels for postmenopausal women are 6.0–54.7 pg/mL.

[c]Normal SHBG levels for women >49 years of age are 17.3–125.0 nmol/L.

TABLE XV

CATEGORICAL CHARACTERISTICS FOR 77 ELIGIBLE POSTMENOPAUSAL WOMEN, GLFCS

Characteristic	Percent of total
Age 46–50 years	3.9
51–60 years	54.6
61–70 years	37.7
71–79 years	3.9
Alcohol consumption ≥12 drinks/year	58.4
BMI ≥30 kg/m^2 (obese)	27.3
Cigarette smoking	3.9
Caucasian	97.4
Antidiabetics	6.5
Thyroid hormones	19.5
Oophorectomy	29.9
Hysterectomy	31.2
Referent[a]	15.6
∑Mono-*ortho* PCBs <0.20 ng/g[b]	60.0

[a]Referents were eligible to participate in the study if they had not eaten any Great Lakes sport fish during the previous 12 months and had not eaten more than six sport fish meals in any year since 1970.

[b]∑Mono-*ortho* PCBs were dichotomized as <LOD and ≥LOD.

2. Bivariate analyses

In unadjusted analyses (data not shown), BMI was higher in participants taking thyroid hormones while FSH was higher in participants not taking thyroid hormones, although both associations were of borderline significance. Table XVIa and XVIb shows significant positive relationships among the PCB groupings. Follicle-stimulating hormone was inversely associated with ∑Wolff estrogenic PCBs (r=-.19) and ∑Wolff anti-estrogenic PCBs (r=-.21), while SHBG-E2 was positively associated with ∑Wolff estrogenic PCBs (r=.21); however, associations were of borderline significance. Estradiol, SHBG, and the ratio of FSH to estradiol (FSH:E2) were not significantly associated with PCBs. All PCB groupings were significantly and positively associated with age, while ∑Wolff estrogenic PCBs were significantly and negatively associated with BMI.

3. Pearson's partial correlation coefficients

In analyses adjusted for age, BMI, and lipids (Table XII), FSH was significantly and negatively associated with both ∑PCB 180 (r=-0.26) and ∑Wolff estrogenic PCBs (r=-.26). Similar results were found for FSH:E2. Estradiol was positively and significantly associated with ∑Wolff estrogenic PCBs (r=.23), while SHBG was significantly and negatively associated with PCB 163/180 (r=-.26) and ∑non-dioxin-like PCBs (r=-.25). No significant associations were found between PCBs and SHBG-E2.

4. Effect modification

Table XVIII, Appendix C shows *p*-values for significant or borderline significant interactions of potential effect modifiers obtained from hierarchical regression models. To help understand the pattern of interaction, we presented dichotomous estimates and also estimates centered at the 25th, 50th, and 75th percentiles of continuous potential effect modifiers (Table XVIII, Appendix C). We found inverse associations between FSH and both PCB 180 and ∑Wolff estrogenic PCBs. Effects were stronger both in participants taking antidiabetics and participants not taking thyroid hormones. We also noted limited evidence that alcohol consumption modified the effect of PCB 163/138 on FSH, with a borderline significant inverse association among participants with low consumption status. We found a positive association between estradiol and ∑Wolff estrogenic PCBs. Effects were stronger for associations of estradiol and PCBs in obese participants. Inverse associations between SHBG and both PCB 163/138 and ∑non-dioxin-like PCBs were found, with stronger effects in participants not taking antidiabetics. We also noted limited evidence that age modified the effect of ∑mono-*ortho* PCBs on SHBG, with a significant and stronger inverse association among younger postmenopausal participants. Sex hormone-binding globulin-bound estradiol was not significantly associated with PCBs. However, positive associations were noted between PCBs and SHBG-E2 for participants with normal and overweight BMI and negative associations were noted for obese participants. In general, associations conditioned on BMI were not significantly associated.

TABLE XVIa

PEARSON'S CORRELATION COEFFICIENTS FOR 77 ELIGIBLE POSTMENOPAUSAL WOMEN, GLFCS

	Ln∑PCBs	Ln∑NDL	Ln∑CE	Ln∑WE	Ln∑WA
Ln∑PCBs		0.998**	0.97**	0.93**	0.74**
Ln∑NDL	0.998**		0.98**	0.93**	0.70**
Ln∑CE	0.97**	0.98**		0.86**	0.70**
Ln∑WE	0.93**	0.93**	0.86**		0.66**
Ln∑WA	0.74**	0.70**	0.70**	0.66**	
FSH	-0.17	-0.15	-0.13	-0.19*	-0.21*
LnE2	-0.025	-0.033	-0.026	0.090	0.0031
SHBG	-0.16	-0.17	-0.16	-0.15	-0.068
SHBG-E2	0.19	0.19	0.17	0.21*	0.12
FSH:E2	-0.090	-0.073	-0.050	-0.18	-0.15
Age	0.39**	0.39**	0.41**	0.33**	0.30**
BMI	-0.18	-0.20*	-0.13	-0.25**	0.077
Lipids	0.078	0.070	0.052	0.13	0.049
LnCR	-0.059	-0.068	-0.049	-0.098	0.064
LnGGT	0.16	0.16	0.15	0.15	0.053

TABLE XVIb

	FSH	LnE2	SHBG	SGBG-E2	FSH:E2	Age	BMI	Lipids	LnCRP	LnGGT
Ln∑PCBs	-0.17	-0.025	-0.16	0.19	-0.090	0.39**	-0.18	0.078	-0.059	0.16
Ln∑NDL	-0.15	-0.033	-0.17	0.19	-0.073	0.39**	-0.20*	0.070	-0.068	0.16
Ln∑CE	-0.13	-0.026	-0.16	0.17	-0.050	0.41**	-0.13	0.052	-0.049	0.15
Ln∑WE	-0.19*	0.090	-0.15	0.21*	-0.18	0.33**	-0.25**	0.13	-0.098	0.15
Ln∑WA	-0.21*	0.0031	-0.068	0.12	-0.15	0.30**	0.077	0.049	0.064	0.053
FSH		-0.080	0.070	0.11	0.76**	-0.16	-0.32**	0.050	-0.095	0.16
LnE2	-0.080		-0.091	0.060	-0.66**	-0.20*	0.29**	0.20*	0.0019	-0.18
SHBG	0.070	-0.091		0.51**	0.098	0.086	-0.10	-0.080	0.15	-0.057
SHBG-E2	0.11	0.060	0.51**		0.051	0.26**	-0.27**	-0.22*	-0.057	-0.087
FSH:E2	0.76**	-0.66**	0.098	0.051		0.064	-0.39**	-0.13	-0.030	0.20
Age	-0.16	-0.20*	0.086	0.26**	0.064		-0.034	-0.19	0.092	0.057
BMI	-0.32**	0.29**	-0.10	-0.27**	-0.39**	-0.034		-0.010	0.23*	-0.22*
Lipids	0.050	0.20*	-0.080	-0.22*	-0.13	-0.19	-0.010		0.051	0.13
LnCR	-0.095	0.0019	0.15	-0.057	-0.030	0.092	0.23*	0.051		0.062
LnGGT	0.16	-0.18	-0.057	-0.087	0.20	0.057	-0.22*	0.13	0.062	

* 0.05≤ *p*<.10
** *p*<.05
NDL=non-dioxin-like PCBs
CE=Cooke estrogenic PCBs
WE=Wolff estrogenic PCBs
WA=Wolff anti-estrogenic PCBs
E2=estradiol
FSH:E2=ratio of FSH to estradiol

TABLE XVII

PEARSON'S PARTIAL CORRELATION COEFFICIENTS FOR ASSOCIATIONS OF PCBS WITH FSH, ESTRADIOL, SHBG, SHBG-E2, AND FSH:E2 AMONG 77 ELIGIBLE POSTMENOPAUSAL WOMEN, GLFCS

	FSH (mIU/mL)		LnEstradiol (pg/mL)		SHBG (nmol/L)		SHBG-E2 (%)		FSH:E2	
POP	**r[a]**	***p*-value[b]**	**r[a]**	***p*-value[b]**	**r[a]**	**p-value[b]**	**r[a]**	***p*-value[b]**	**r[a]**	***p*-value[b]**
Sample size	77		77		68		68		77	
LnPCB 163/138 (ng/g)	-0.07	0.54	0.075	0.52	-0.26	**0.03**	0.028	0.82	-0.08	0.50
LnPCB 132/153/105 (ng/g)	-0.12	0.31	0.09	0.44	-0.22	0.08	0.06	0.63	-0.13	0.28
LnPCB 180 (ng/g)	-0.26	**0.03**	0.16	0.18	-0.18	0.15	0.14	0.28	-0.28	**0.02**
∑Mono-*ortho* PCBs (ng/g)	-0.11	0.37	0.056	0.64	-0.091	0.47	0.11	0.37	-0.14	0.22
Ln∑PCBs (ng/g)	-0.20	0.09	0.092	0.44	-0.24	0.06	0.075	0.55	-0.20	0.08
Ln∑Non-dioxin-like PCBs (ng/g)	-0.19	0.11	0.088	0.46	-0.25	**0.048**	0.071	0.58	-0.19	0.11
Ln∑Cooke estrogenic PCBs (ng/g)	-0.13	0.29	0.082	0.49	-0.23	0.07	0.054	0.67	-0.13	0.27
Ln∑Wolff estrogenic PCBs (ng/g)	-0.26	**0.02**	0.23	**0.045**	-0.23	0.07	0.11	0.40	-0.33	**0.004**
∑Wolff anti-estrogenic PCBs (ng/g)	-0.16	0.18	0.024	0.84	-0.087	0.49	0.088	0.49	-0.14	0.23

[a]Partial correlation coefficients adjusted for age, BMI, and lipids.

[b]*P*-values in bold are statistically significant at $p<0.05$.

r=Correlation coefficient

When PCBs were assessed according to classification schemes, associations of anti-estrogenic and/or dioxin-like PCB groupings with estradiol and SHBG-E2 generally depended on BMI, with stronger effects in obese participants. We found little evidence of effect modification of associations of PCBs with the ratio of FSH to estradiol (data not shown). Selected associations of PCBs and hormones by potential effect modifiers are presented in Figures 4 and 5.

5. Sensitivity analyses

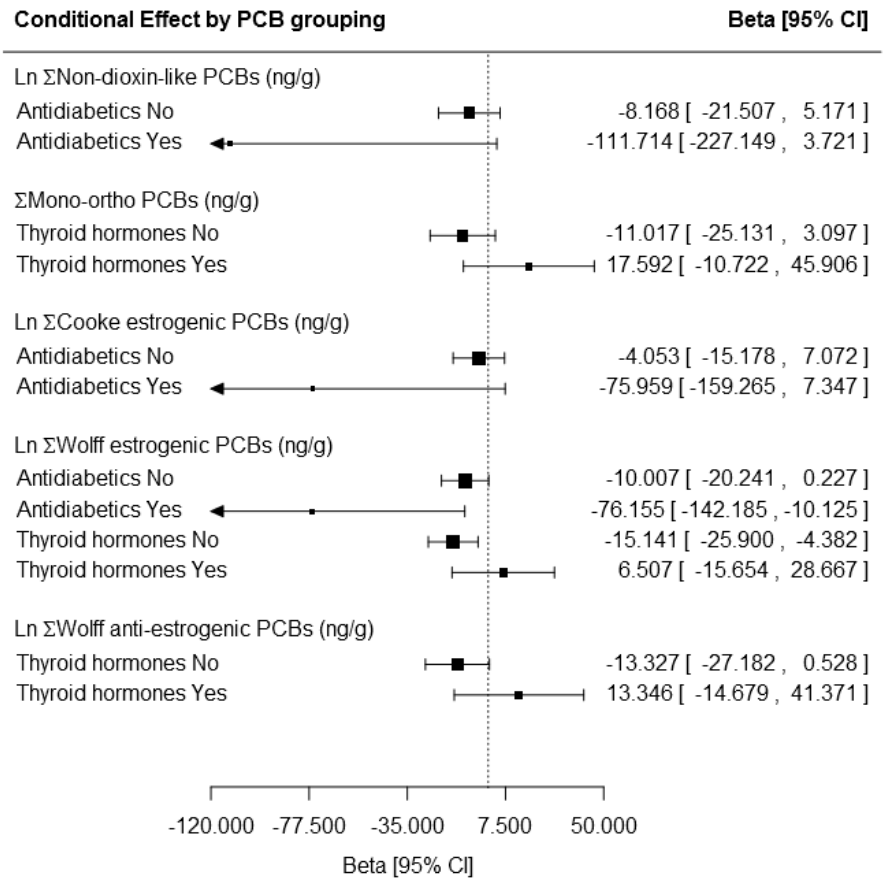
To determine if associations of PCBs and hormones were affected by an extreme FSH measurement, we excluded a participant with an FSH level equal to 177.2 mIU/mL. Some but not all significant associations between PCBs and hormones remained. The inverse association between PCB 180 and FSH did not remain significant. In addition, the associations between ∑Wolff estrogenic PCBs and FSH, ∑non-dioxin-like PCBs and SHBG, and PCB 180 and FSH:E2 became borderline significant. There were five participants aged 60 years and older with FSH values below the normal range for postmenopausal women. Excluding these participants did not change the results. Associations were also reestimated using PCB congeners with results below the LOD calculated as the LOD divided by the square root of two but this reestimation did not affect our findings. Bilateral oophorectomy in postmenopausal women was shown to affect steroid hormone levels (Laughlin et al., 2000; Korse et al., 2009). Therefore, associations of PCBs with hormones were repeated excluding 23 postmenopausal participants who reported oophorectomy. Associations of PCBs with FSH, estradiol, and SHBG were weaker (p>.05 for associations of both PCB 180 and ∑Wolff estrogenic PCBs with FSH, ∑Wolff estrogenic PCBs with estradiol, and both PCB 163/138 and ∑non-dioxin-like PCBs with SHBG; data not shown). However, several associations of PCBs with SHBG-E2 became borderline significant ($0.05 \leq p < .10$ for PCB 132/153/105, PCB 180, ∑mono-*ortho* PCBs, ∑non-dioxin-like PCBs, and ∑Wolff estrogenic PCBs; data not shown). Associations of PCBs with hormones were also estimated using lipid-standardized PCBs. Lipid-standardized results adjusted for age and BMI were similar to non-standardized results adjusted for age, BMI, and lipids.

C. Discussion

1. Hormones and binding proteins

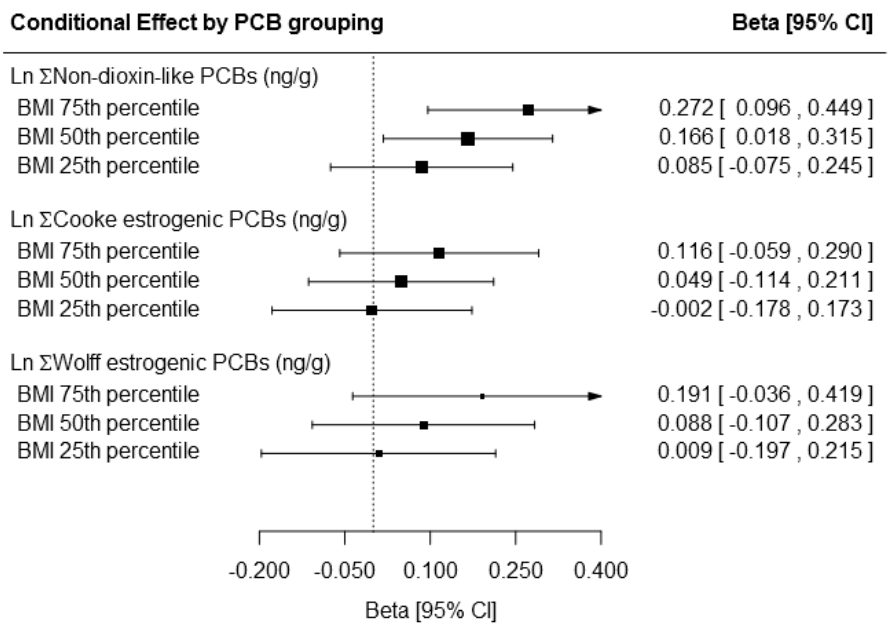
In this study of postmenopausal women participating in the GLFCS who were not taking glucocorticoids or sex hormones, we found inverse associations between FSH and both PCB 180 and ∑Wolff estrogenic PCBs. Similar findings were obtained for FSH:E2. Effects of PCBs on FSH were stronger both in participants taking antidiabetics and participants not taking thyroid hormones. We found a positive association between estradiol and ∑Wolff estrogenic PCBs. Effects were stronger for associations of estradiol and PCBs in obese participants. Inverse associations between SHBG and both PCB 163/138 and ∑non-dioxin-like PCBs were found, with stronger effects in participants not taking antidiabetics. Sex hormone-binding globulin-bound estradiol was not significantly associated with PCBs. However, positive associations were found between PCBs and SHBG-E2 for

participants with normal and overweight BMI, and negative associations were found for obese participants. When PCBs were assessed according to classification schemes, associations of estrogenic and/or non-dioxin-like PCB groupings with estradiol and SHBG-E2 depended on BMI, with stronger effects in obese participants.



Box size indicates the precision of the estimate; larger boxes signify less variance.
A confidence interval (CI) that extends beyond the plot domain is indicated by an arrow.

Figure 4. Selected associations of PCBs with FSH by potential effect modifiers for 77 eligible postmenopausal women, GLFCS.



Box size indicates the precision of the estimate; larger boxes signify less variance.
A confidence interval (CI) that extends beyond the plot domain is indicated by an arrow.

Figure 5. Selected associations of PCBs with estradiol by potential effect modifiers for 77 eligible postmenopausal women, GLFCS.

In general, findings of human studies of the effects of PCBs on steroid hormones, gonadotrophins, or binding proteins have been inconsistent. A lack of association between PCBs and hormones in previous investigations could be indicative of a threshold of bioavailable estrogen necessary impacts on circulating hormones, although we found no evidence of effect modification by estradiol for the associations between PCBs and FSH, SHBG, SHBG-E2, and FSH:E2. Further, the effects of mixtures of PCB congeners may depend on the population, endpoint, and dose. Because these data were obtained from a cohort of frequent and infrequent Great Lakes fish consumers, PCB levels in our report were somewhat higher than background exposures in the general US population (Turyk et al., 2012).

The current investigation is consistent with a previous investigation in postmenopausal women with occupational PCB exposures where total PCBs were significantly and inversely associated with FSH and SHBG (Persky et al., 2011). Occupational exposures to PCBs were not associated with FSH in men from the same study (Persky et al., 2012). Most investigations representing a range of age and exposure levels in men have not found associations of PCBs with estradiol, SHBG, or FSH. In healthy men from the West Indies, PCB 153 was positively associated with androstenedione and estrone levels but was not related to FSH (Emeville et al., 2013). In men from a United States fertility clinic, PCB 118 was inversely associated with SHBG but was not related to FSH (Ferguson et al., 2012). In Inuits and three European cohorts, positive associations of PCB 153 were seen with SHBG in some but not all groups (Giwercman et al., 2006). In a study of young Swedish men from the general population, PCB 153 was inversely associated with the testosterone-to-SHBG ratio but was not related to FSH (Richthoff et al., 2003). Polychlorinated biphenyl 153 was not associated with FSH in men exposed to high levels of POPs through fish consumption (Hagmar et al., 2001). Previous investigations of men from the GLFCS have shown that PCBs were inversely related with SHBG-bound testosterone but not estrone sulfate, FSH, or SHBG (Persky et al., 2001). In a subgroup of men from the same cohort, models that accounted for both dioxin-like chemicals and PCBs predicted SHBG-bound testosterone (Turyk et al., 2006). In general, research has shown an association between PCB exposure and SHBG-related measures, suggesting that PCBs may affect the binding of estrogen. An increasing number of animal studies give biological plausibility to PCB-induced effects of hormone homeostasis. Animal and in vitro investigations have shown associations between PCB exposure and FSH levels, although results have been inconsistent and differed by PCB congener or aroclor (Desaulniers et al., 1999; Wade et al., 2002; Oskam et al., 2005; Uslu et al., 2013).

We found that some estrogenic and/or non-dioxin-like PCB groupings and congeners were negatively associated with FSH and SHBG. Decreased FSH indicates a normal pituitary response of negative feedback resulting from exogenous estrogenicity. Positive associations were found for the associations of PCBs and estradiol, but only the effect of ∑Wolff estrogenic PCBs on estradiol reached statistical significance. The differential effects of estrogenic and/or non-dioxin-like PCB

groupings and congeners on hormones could potentially be attributed to their similarity to estradiol in terms of chemical structure and the capacity to bind and activate estrogen receptors that initiate transcription (Cooke et al., 2001). In addition, some PCBs may inhibit estrogen sulfotransferase (Kester et al., 2002). We also found that PCBs were negatively associated with SHBG, suggesting that PCBs may affect the level of the binding protein. However, we found no effect of PCBs on SHBG-E2.

2. Effect modification

Associations of PCBs with FSH depended on thyroid hormone use, with inverse associations in participants not taking these medications. Interactions, however, were of borderline significance. The metabolism of both androgens and estrogens is altered in hypothyroidism. "With respect to estradiol and estrone, hypothyroidism favors metabolism of these steroids via 16α-hydroxylation over 2-oxygenation, resulting in increased formation of estriol at the expense of 2-hydroxyestrone and its derivative, 2-methohyestrone" (Doufas and Mastorakos, 2000). Sex hormone-binding globulin in circulation is also decreased, resulting in decreased levels of estradiol, but the bioavailable fraction is increased (Brenta et al., 1999). Participants taking thyroid hormones, however, had higher mean BMI and lower mean FSH, although these differences were of borderline significance (data not shown). Increased BMI (Kopelman et al., 1981) and decreased FSH (Cosma et al., 2008; Shaw et al., 2010) are related to estrogenicity.

Inverse associations were found for PCB groupings and FSH in participants who were taking antidiabetic medications. In addition, PCBs were inversely related to SHBG in participants who were not taking antidiabetics. Despite the small percentage of postmenopausal women taking these medications in the current study (6.5%), significant inverse associations of PCBs with FSH for postmenopausal women taking antidiabetic medications were not attributed to outliers based on scatter plots (data not shown). It is not clear if diabetes itself might decrease the metabolism of PCBs, although differences in PCB trends for those with and without diabetes have not been observed in the literature (Michalek et al., 2003; Turyk et al., 2009). Steroid hormones (Ding et al., 2006) or environmental chemicals acting as steroid hormones (Swedenborg et al., 2009) may increase the risk of developing diabetes. In the current study, exposures and hormones were not associated with use of antidiabetic medication. Unexpectedly, diabetes was not associated with BMI in the current study. Type 1 diabetes is considered to be of autoimmune origin, whereas type 2 diabetes is primarily associated with obesity and metabolic syndrome, although disease can occur independent of obesity (Taylor et al., 2013). Biologic mechanisms by which estrogenic PCB exposures could decrease FSH in diabetics have not been delineated but could relate to the estrogen-dependent ratio of alpha to beta estrogen receptors (Barros et al., 2006).

Inverse associations were found for PCB groupings and estradiol in obese postmenopausal participants. In addition, positive associations were noted between PCBs and SHBG-E2 for participants with normal and overweight BMI and negative associations were noted for obese participants. These associations conditioned on BMI at the 25th, 50th, and 75th percentile were not generally significant. There is evidence that body composition may play an important role in steroid hormones and SHBG concentrations. In general, the aromatization of androstenedione to estrone in adipose tissue correlates positively with weight (Kopelman et al., 1981; Bulun et al., 1994). Conversely, inverse correlations have been reported between BMI and SHBG for postmenopausal women, resulting in increased bioavailable estradiol (Cauley et al., 1989). Recent evidence suggests that PCBs may cause perturbations in endogenous hormonal regulation that affect weight gain.

3. Limitations and strengths

The present report has a number of limitations. The cross-sectional design of the study does not allow us to establish a temporal relationship of PCB exposures with changes in steroid, gonadotropin, or binding protein levels. In addition, findings may be affected by a lack of power to detect relationships between PCBs and outcomes due to limited sample size. We adjusted for age, BMI, and lipids, but there are other important potential confounders for which we have not controlled. Antidiabetic medication use did not allow us to distinguish between use of TZDs, a class of antidiabetic drugs that may inhibit estrogen synthesis (Seto-Young et al., 2011). Additional PCB congener measurements for complete groupings might have helped to better elucidate mechanisms related to associations between PCB groupings and hormones. Estrogenic and anti-estrogenic classes of PCB congeners can express varied and sometimes conflicting effects (Warner et al., 2012). Finally, the findings might be due to chance, as multiple comparisons were made in the statistical analysis.

Despite the limitations, our investigation has a number of strengths. Our investigation adds to the epidemiologic literature and increases the confidence that associations we have reported are not the product of chance due to some similar trends and consistency with a previous investigation. To our knowledge, only one other study has evaluated the association of PCBs on hormones in postmenopausal women. We also investigated the associations using continuous data and centered potential effect modifiers to ensure we did not further limit the power of our analysis. Finally, multiple exposure groupings based on proposed mechanism of action, confounders, and effect modifiers were evaluated.

D. Conclusion

This investigation confirms a previous study in occupationally exposed postmenopausal women that found inverse associations of PCBs with FSH and SHBG. Future studies should investigate the mechanisms underlying the observed interactions with factors potentially affecting estrogen profile.

VI. CONCLUSIONS

Our analyses added significantly to previous investigations of associations of POPs on hormones and BMD among postmenopausal women in two ways. First, this report is focused on the relationships of BMD and hormones with POPs including dioxin-like TEQs, individual PCB congeners, ∑PCBs, and PCB congeners grouped into classes with structural similarity or mechanism of action. Second, we examined the hypothesis that associations of POPs with both hormones and BMD were modified by factors that may affect endogenous hormonal profile.

Findings were not always consistent across studies, which may be attributed to important differences between participants from the NHANES and the GLFCS. Postmenopausal women in the GLFCS were majority Caucasian, older, and leaner. Fewer participants were smokers and taking antidiabetics, while a higher proportion consumed alcohol on a regular basis and were taking thyroid hormones. C-reactive protein and GGT levels were relatively low and PCB levels were notably higher compared with the NHANES (Table XIX).

Our findings are somewhat consistent with previous investigations suggestive of an estrogenic effect of POP body burdens. In the current study, there was some but not consistent evidence of a dose-response increase in BMD with exposure to both estrogenic and/or non-dioxin-like POPs and anti-estrogenic and/or dioxin-like POPs among 603 NHANES participants. We also found inverse associations LH but not FSH, with exposure to anti-estrogenic and/or dioxin-like POPs among 89 participants in NHANES. Gamma-glutamyl transferase, a potential confounder, attenuated these associations. Finally, we found inverse associations of FSH and SHBG with exposure to estrogenic and/or non-dioxin-like PCBs among 77 women participating in the GLFCS. Inverse associations of PCBs with FSH suggest that PCBs may be independently estrogenic and are acting through negative feedback mechanisms of the HPG axis.

Gamma-glutamyl transferase was determined to be an important potential confounder for associations between POPs and hormones in NHANES but not for the GLFCS. Gamma-glutamyl transferase, a marker of oxidative stress and an enzyme catalyzing the extracellular degradation of glutathione (Turgut and Tandogan, 2011), was considerably higher among NHANES participants. In the NHANES investigation and in previous studies, GGT levels were found to be positively associated with CRP (Lee et al., 2003b) and POP exposures (Lee et al., 2008). Gamma-glutamyl transferase was also inversely related to FSH and LH in the NHANES investigation. Mechanisms by which GGT could affect gonadotropin hormones are not clear and warrant further investigation.

We observed increased BMD with exposure to both estrogenic and/or non-dioxin-like POPs and anti-estrogenic and/or dioxin-like POPs. Both androgens and estrogens play an important role in bone physiology. Lack of more robust associations between POPs and BMD may be related to low general population exposure levels found in NHANES. In addition, the 2003–2004 study cycle did not have FSH measurements for the identification and exclusion of potentially misclassified postmenopausal women; however, for eligible participants with FSH measures in 1999–2000 and 2001–2002, six out of 93 (6.5%) had FSH levels below the reference range.

Associations were enhanced by potential effect modifiers related to both direct and indirect estrogenicity including hypothyroidism, obesity, and the obesity-related conditions diabetes and inflammation. The metabolism of both androgens and estrogens is affected in hypothyroidism. Androgen production is decreased and the metabolism of testosterone is shifted toward etiocholanolone rather than androsterone. With respect to estradiol and estrone, hypothyroidism favors metabolism of these steroids via the more estrogenic 16α-hydroxylation rather than 2-hydroxylation. Sex hormone-binding globulin in circulation is also decreased, resulting in decreased levels of estradiol, but the bioavailable fraction is increased. Because aromatization of androgen precursors to estrogen occurs predominately in peripheral fat, obese women have higher estrogen levels and are therefore at lower risk of diseases related to estrogen deficiency, such as osteoporosis. Obesity is also associated with decreased SHBG, resulting in an increased fraction of bioavailable estrogen. Obesity is a major risk factor influencing the risk of type 2 diabetes and has been related to elevated CRP.

Overall, effect modification by obesity and obesity-related conditions could be effects not only on aromatase but also on decreased liver production of SHBG and, in turn, increased bioavailable estrogen through direct effects by POPs. Higher levels of PCBs, such as in the GLFCS, may directly decrease liver production of SHBG. Hypothyroidism may also affect estrogen levels through 16α-hydroxylation and decreased SHBG, but may also act through other mechanisms not addressed in these analyses.

Our investigations focused on the relationships of BMD and hormones with POP congeners grouped into classes with similar structure or mode of action. Associations were observed with anti-estrogenic and/or dioxin-like PCB groupings in NHANES and estrogenic and/or non-dioxin-like PCB groupings in the GLFCS (Table XX). Although we are unable to compare PCB congener profiles across studies, lower chlorinated PCBs are less persistent and are suggested to have estrogenic properties. Associations with estrogenic PCBs found in the GLFCS may be attributed to higher overall exposure levels and possibly to concurrent environmental chemical exposure through fish consumption. Great Lakes charter boat captains and their spouses were recruited for the GLFCS because they consumed Great Lakes sport-caught fish (Turyk et al., 2012). Follicle-stimulating

hormone was the only hormone available in both NHANES and GLFCS. Differences between studies may be related to the beneficial effects of omega 3 fatty acids in fish (Cao et al., 2012), differences in POP body burden profiles, and/or assays used for hormone and POP measurements.

Some of our findings are inconsistent with previous investigations suggesting an estrogenic effect of POP body burdens. We found inverse associations of LH with exposure to anti-estrogenic and/or dioxin-like POPs in NHANES. Possible mechanisms to explain this observation include cross-talk between the AhR and estrogen receptors and a previous investigation that observed up-regulation of genes coding for aromatase and estrogen receptor beta with exposure to anti-estrogenic and dioxin-like PCBs (Warner et al., 2012). We found positive associations between PCBs and estradiol, but associations were mostly nonsignificant. Mechanisms by which POPs could affect estrogenicity without direct effects on estradiol could relate to up-regulation of genes for estrogen receptors, alpha and beta, and/or direct effects on hormone receptors. Chemicals in the environment acting as steroid hormones due to their structural similarity may bind to estrogen receptors and stimulate transcription of proteins known to mediate the effects of estrogen. We did not observe effect modification for the associations of PCBs with FSH, SHBG, and SHBG-E2 by estradiol. In postmenopausal women, estrone becomes the predominant form of estrogen, although it is weaker than estradiol, perhaps explaining the lack of effect modification by estradiol.

Our findings expand upon an investigation of associations of POPs and increased BMD with evidence that associations were positive among older women with FM and inversely associated among older women with low FM (Cho et al., 2011). In animal studies, the effects of POPs on BMD appeared to be modified by estrogen status. Investigations in rats exposed to the dioxin-like PCB 126 showed impaired bone strength and composition, and effects were modulated depending on the estrogen profile of the animal. Our BMD study is consistent with a previous investigation showing estrogen supplementation in rats exposed to PCB 126 modulated effects on bone tissue (Lind et al., 2004). Our results also expand upon a previous investigation of occupational PCB exposures and decreased FSH and SHBG in postmenopausal women (Persky et al., 2011) with high PCB exposures and an age range comparable to those found in the GLFCS.

The present report has a number of limitations. The cross-sectional design of each investigation does not allow us to establish a temporal relationship. Body mass index may be a poor measure of body fat. Estrogenic and anti-estrogenic classes of PCB congeners may express conflicting effects (Warner et al., 2012). Findings might be due to chance, as multiple comparisons were made in the statistical analyses and findings may be affected by lack of power to detect associations between POPs and hormones due to limited sample size. Other limitations include the limited number of hormones studied, the potential for unmeasured confounders, and results that may not be generalizable to populations other than postmenopausal women.

These investigations help to establish a pattern of endocrine-disrupting effects by POPs among postmenopausal women, especially those with conditions related to peripheral estrogenicity. Future investigations should include evaluation of effect modification by estrone, the predominant form of estrogen in postmenopausal women, as well as the major metabolites of estradiol and estrone: estriol and catechol estrogens and other steroid hormones. Measures of waist-to-hip ratio or other measures of centrally distributed body fat should be assessed. Further investigations should also include evaluation of effect modification by CRP and diabetes using other markers of inflammation and hemoglobin A1c. Finally, elucidation of the complex relationship of steroid and gonadotropin hormones with thyroid hormones and GGT await further investigation.

TABLE XIX

COMPARISON OF CHARACTERISTICS AMONG ELIGIBLE POSTMENOPAUSAL WOMEN, NHANES 1999–2002 AND THE GLFCS

	NHANES	GLFCS
Characteristic	**Percent of total, mean, or geometric mean**	**Percent of total, mean, or geometric mean**
Sample size	89	77
Alcohol consumption ≥12 drinks/year	52.8	58.4
BMI ≥30 kg/m^2 (obese)	46.1	37.3
Cotinine >10 ng/mL or cigarette smoking	33.7	3.9
Race/ethnicity Caucasian	36.0	97.4
African American	24.7	0.0
Other	39.3	2.6
Antidiabetics	18.0	6.5
Thyroid hormones	11.2	19.5
Oophorectomy	11.2	29.9
Hysterectomy	24.7	31.2
Ln∑PCBs (ng/g)	1.2	2.4
Ln PCB 180 (ng/g)	0.22	0.32
∑Wolff estrogenic PCBs (ng/g)	0.11	0.24
FSH (mIU/mL)	68.6	64.2
Age (years)	54.3	59.3
BMI (kg/m^2)	29.5	27.9
LnLipids (mg/dL)	675.1	707.2
LnCRP (mg/dL)	0.32	0.23
LnGGT (U/L)	27.0	17.9

TABLE XX

COMPARISON OF PEARSON'S PARTIAL CORRELATION COEFFICIENTS OF SELECTED PCBs WITH FSH AMONG ELIGIBLE POSTMENOPAUSAL WOMEN, NHANES 1999–2002 AND THE GLFCS

	r[a] FSH (mIU/mL)	
Characteristic	**NHANES**	**GLFCS**
Sample size	89	77
Ln∑PCBs (ng/g)	-0.017	-0.20*
LnPCB 180 (ng/g)	-0.018	-0.26**
Ln∑Wolff estrogenic PCBs (ng/g)	-0.084	-0.26**

* $0.05 \leq p < 0.10$

** $p < 0.05$

[a]Partial Pearson's correlation coefficients adjusted for age, BMI, and lipids.

r=Correlation coefficient

APPENDICES

APPENDIX A
TABLE VI

INTERACTION TERM *P*-VALUES AND ASSOCIATIONS OF POPs WITH BMD BY POTENTIAL EFFECT MODIFIERS IN 603 ELIGIBLE POSTMENOPAUSAL WOMEN, NHANES 1999–2004

POP, BMD site, potential effect modifier	Interaction *p*-value[a]	Beta 25th Percentile or Beta No	*p*-value[b]	Beta 50th Percentile or Beta Yes	*p*-value[b]	Beta 75th Percentile	*p*-value[b]
Ln ∑TEQs (pg/g)[c]							
Left arm BMD (g/cm^2)							
Age	0.008	0.0079	0.06	0.0019	0.52	-0.0057	0.10
Left leg BMD (g/cm^2)							
Age	0.004	0.022	**0.004**	0.012	**0.03**	0.00061	0.91
Thyroid hormones	0.07	0.0079	0.13	0.033	**0.03**		
Thoracic spine BMD (g/cm^2)							
Thyroid hormones	0.04	0.0012	0.85	0.028	**0.02**		
Lumbar spine BMD (g/cm^2)							
BMI	0.06	-0.00090	0.93	0.0066	0.46	0.015	0.12
Pelvis BMD (g/cm^2)							
Age	0.02	0.026,	0.07	0.011	0.25	-0.0078	0.37
Thyroid hormones	0.04	0.0036	0.68	0.045	**0.04**		
Ln∑PCBs (ng/g)[d]							
Left arm BMD (g/cm^2)							
BMI	0.02	-0.0018	0.62	0.0029	0.38	0.0084	**0.05**
LnCRP	0.03	-0.00012	0.97	0.0036	0.30	0.0087	0.06
Left leg BMD (g/cm^2)							
BMI	0.02	0.0033	0.61	0.010	0.09	0.019	**0.01**
LnCRP	0.04	0.0059	0.37	0.011	0.07	0.019	**0.01**
Thoracic spine BMD (g/cm^2)							
Thyroid hormones	0.07	0.0048	0.041	0.027	**0.02**		
Lumbar spine BMD (g/cm^2)							
BMI	0.01	0.0014	0.87	0.011	0.15	0.022	**0.01**
Antidiabetics	0.08	0.0061	0.46	0.036	**0.03**		
Pelvis BMD (g/cm^2)							
Age	0.07	0.015	0.27	0.0046	0.61	-0.0084	0.28
BMI	0.02	-0.0029	0.75	0.0071	0.42	0.019	0.09
LnCRP	0.09	0.0015	0.88	0.0079	0.38	0.017	0.11
Thyroid hormones	0.04	0.0021	0.81	0.039	**0.05**		

APPENDIX A (continued)

POP, BMD site, potential effect modifier	Interaction *p*-value[a]	Beta 25th Percentile or Beta No	*p*-value[b]	Beta 50th Percentile or Beta Yes	*p*-value[b]	Beta 75th Percentile	*p*-value[b]
Ln∑Non-dioxin-like PCBs (ng/g)[e]							
Left arm BMD (g/cm^2)							
BMI	0.01	0.00070	0.88	0.0043	0.22	0.010	**0.02**
LnCRP	0.02	0.00085	0.80	0.0046	0.18	0.0097	**0.03**
Left leg BMD (g/cm^2)							
BMI	0.006	0.0045	0.54	0.013	0.07	0.022	**0.005**
LnCRP	0.02	0.0077	0.27	0.013	0.06	0.020	**0.009**
Lumbar spine BMD (g/cm^2)							
BMI	0.02	0.0051	0.54	0.15	**0.05**	0.026	**0.004**
LnCRP	0.09	0.0093	0.21	0.015	**0.05**	0.023	**0.02**
Pelvis BMD (g/cm^2)							
Age	0.06	0.022	0.14	0.010	0.32	-0.0047	0.60
BMI	0.02	0.0029	0.80	0.014	0.20	0.027	**0.02**
LnCRP	0.09	0.0080	0.47	0.014	0.19	0.022	0.07
Ln∑Mono-*ortho* PCBs (ng/g)[d]							
Left arm BMD (g/cm^2)							
BMI	0.03	-0.0020	0.56	0.0021	0.44	0.0068	**0.04**
LnCRP	0.006	-0.00080	0.79	0.0032	0.22	0.0088	**0.008**
Antidiabetics	0.01	0.00033	0.91	0.017	**0.006**		
Left leg BMD (g/cm^2)							
BMI	0.09	0.0057	0.36	0.010	0.06	0.016	**0.01**
LnCRP	0.01	0.0060	0.33	0.012	**0.03**	0.019	**0.001**
Antidiabetics	0.02	0.0069	0.22	0.034	**0.004**		
Thoracic spine BMD (g/cm^2)							
Thyroid hormones	0.02	0.0047	0.38	0.034	**0.003**		
Lumbar spine BMD (g/cm^2)							
BMI	0.06	0.0056	0.50	0.012	0.15	0.019	**0.04**
LnCRP	0.06	0.0080	0.35	0.014	0.10	0.021	**0.03**
Pelvis BMD (g/cm^2)							
LnCRP	0.07	0.0070	0.46	0.013	0.10	0.022	**0.01**
Antidiabetics	0.04	0.0083	0.35	0.043	**0.006**		

APPENDIX A (continued)

POP, BMD site, potential effect modifier	Interaction *p*-value[a]	Beta 25th Percentile or Beta No	*p*-value[b]	Beta 50th Percentile or Beta Yes	*p*-value[b]	Beta 75th Percentile	*p*-value[b]
Thyroid hormones	0.03	0.0083	0.31	0.047	**0.02**		,
Ln∑Dioxin-like PCBs (ng/g)[d]							
Left arm BMD (g/cm^2)							
BMI	0.08	-0.0017	0.65	0.0018	0.55	0.0058	0.10
LnCRP	0.02	-0.00078	0.81	0.0028	0.32	0.0077	**0.03**
Antidiabetics	0.03	0.00017	0.96	0.015	**0.02**		
Left leg BMD (g/cm^2)							
LnCRP	0.04	0.0060	0.36	0.011	0.06	0.018	**0.005**
Thyroid hormones	0.07	0.0075	0.18	0.035	**0.03**		
Thoracic spine BMD (g/cm^2)							
Thyroid hormones	0.02	0.0045	0.42	0.036	**0.01**		
Pelvis BMD (g/cm^2)							
Antidiabetics	0.03	0.0052	0.56	0.042	**0.01**		
Thyroid hormones	0.02	0.0050	0.55	0.047	**0.03**		
Ln∑Cooke estrogenic PCBs (ng/g)[e]							
Left arm BMD (g/cm^2)							
BMI	0.003	-0.00044	0.90	0.0050	0.12	0.011	**0.006**
LnCRP	0.01	0.0014	0.66	0.0054	0.08	0.011	**0.008**
Antidiabetics	0.04	0.0025	0.45	0.016	**0.01**		
Study cycle[f]	0.07	0.010	**0.01**	0.0046	0.42	0.00030	0.94
Left leg BMD (g/cm^2)							
BMI	0.002	0.0040	0.52	0.013	**0.04**	0.023	**0.002**
LnCRP	0.02	0.0077	0.21	0.013	**0.03**	0.020	**0.004**
Antidiabetics	0.06	0.0078	0.19	0.035	**0.01**		
Lumbar spine BMD (g/cm^2)							
BMI	0.02	0.0087	0.28	0.017	**0.03**	0.027	**0.01**
LnCRP	0.07	0.012	0.13	0.018	**0.03**	0.026	**0.01**
Pelvis BMD (g/cm^2)							
BMI	0.005	-0.00080	0.93	0.011	0.21	0.025	**0.02**
Thyroid hormones	0.09	0.0053	0.52	0.036	0.07		
Ln∑Wolff estrogenic PCBs (ng/g)[e]							

APPENDIX A (continued)

POP, BMD site, potential effect modifier	Interaction *p*-value[a]	Beta 25th Percentile or Beta No	*p*-value[b]	Beta 50th Percentile or Beta Yes	*p*-value[b]	Beta 75th Percentile	*p*-value[b]
Left arm BMD (g/cm^2)							
BMI	0.02	0.00044	0.90	0.0048	0.15	0.010	**0.02**
Left leg BMD (g/cm^2)							
BMI	0.006	0.0032	0.64	0.010	0.10	0.019	**0.01**
LnCRP	0.08	0.0063	0.32	0.011	0.09	0.017	**0.03**
Lumbar spine BMD (g/cm^2)							
BMI	0.02	0.0071	0.41	0.017	**0.04**	0.028	**0.003**
Pelvis BMD (g/cm^2)							
BMI	0.03	0.0014	0.90	0.011	0.30	0.022	0.06
Ln∑Cooke anti-estrogenic PCBs (ng/g)[e]							
Left arm BMD (g/cm^2)							
Antidiabetics	0.02	0.0021	0.56	0.018	**0.01**		
Left leg BMD (g/cm^2)							
Antidiabetics	0.01	0.0085	0.20	0.041	**0.002**		
Thyroid hormones	0.08	0.0035	0.30	0.0059	0.54		
Study cycle[f]	0.05	0.026	**0.003**	0.0062	0.59	0.0083	0.31
Thoracic spine BMD (g/cm^2)							
Antidiabetics	0.04	0.0068	0.24	0.040	**0.01**		
Thyroid hormones	0.02	0.0066	0.23	0.034	**0.003**		
Study cycle[f]	0.07	0.023	**0.003**	0.0088	0.47	0.0020	0.82
Lumbar spine BMD (g/cm^2)							
Antidiabetics	0.05	0.0078	0.39	0.046	**0.01**		
Study cycle[f]	0.07	0.032	**0.03**	0.012	0.37	0.0010	0.93
Pelvis BMD (g/cm^2)							
Antidiabetics	0.07	0.0094	0.41	0.044	**0.02**		
Thyroid hormones	0.01	0.0049	0.59	0.064	**0.01**		
Study cycle[f]	0.09	0.030	**0.01**	0.010	0.58	0.0047	0.72
Ln∑Wolff anti-estrogenic PCBs (ng/g)[c]							
Left arm BMD (g/cm^2)							
Antidiabetics	0.05	0.000047	0.99	0.013	**0.05**		
Left leg BMD (g/cm^2)							

APPENDIX A (continued)

POP, BMD site, potential effect modifier	Interaction *p*-value[a]	Beta 25th Percentile or Beta No	*p*-value[b]	Beta 50th Percentile or Beta Yes	*p*-value[b]	Beta 75th Percentile	*p*-value[b]
Antidiabetics	0.07	0.0068	0.27	0.028	**0.02**		
Thyroid hormones	0.08	0.0073	0.19	0.033	**0.03**		
Thoracic spine BMD (g/cm^2)							
Thyroid hormones	0.02	0.0044	0.44	0.034	**0.01**		
Pelvis BMD (g/cm^2)							
Antidiabetics	0.09	0.0062	0.51	0.0037	**0.05**		
Thyroid hormones	0.02	0.0060	0.48	0.048	**0.02**		
LnPCB 138 (ng/g)[e]							
Left arm BMD (g/cm^2)							
Age	0.03	0.0080	0.07	0.0038	0.26	-0.0016	0.64
BMI	0.004	-0.00077	0.84	0.0050	0.14	0.012	**0.01**
LnCRP	0.01	0.0013	0.68	0.0054	0.10	0.011	**0.01**
Antidiabetics	0.05	0.0032	0.34	0.015	**0.02**		
Left leg BMD (g/cm^2)							
Age	0.02	0.014	**0.04**	0.0083	0.14	0.0012	0.84
BMI	0.002	0.0015	0.80	0.010	0.08	0.020	**0.003**
LnCRP	0.04	0.0055	0.36	0.010	0.07	0.017	**0.01**
Thoracic spine BMD (g/cm^2)							
BMI	0.06	0.0058	0.24	0.012	0.09	0.019	**0.003**
Bisphosphonates	0.001	0.013	**0.01**	-0.056	**0.03**		
Thyroid hormones	0.02	0.0082	0.08	0.040	**0.002**		
Lumbar spine BMD (g/cm^2)							
BMI	0.02	0.0098	0.20	0.018	**0.01**	0.028	**0.001**
LnCRP	0.07	0.013	0.07	0.018	**0.01**	0.026	**0.002**
Bisphosphonates	0.04	0.019	**0.02**	-0.043	0.13		
Pelvis BMD (g/cm^2)							
Age	0.04	0.019	0.09	0.0091	0.27	-0.0039	0.63
BMI	0.01	0.00048	0.95	0.012	0.16	0.025	**0.03**
LnPCB 153 (ng/g)[e]							
Left arm BMD (g/cm^2)							
BMI	0.004	0.00065	0.85	0.0060	0.06	0.012	**0.003**

APPENDIX A (continued)

POP, BMD site, potential effect modifier	Interaction *p*-value[a]	Beta 25th Percentile or Beta No	*p*-value[b]	Beta 50th Percentile or Beta Yes	*p*-value[b]	Beta 75th Percentile	*p*-value[b]
LnCRP	0.02	0.0026	0.43	0.0062	**0.05**	0.011	**0.01**
Left leg BMD (g/cm^2)							
BMI	0.003	0.0055	0.42	0.014	**0.03**	0.024	**0.002**
LnCRP	0.03	0.0090	0.18	0.014	**0.03**	0.021	**0.01**
Thoracic spine BMD (g/cm^2)							
BMI	0.09	0.0059	0.33	0.011	**0.03**	0.018	**0.01**
Lumbar spine BMD (g/cm^2)							
BMI	0.01	0.0072	0.38	0.017	**0.03**	0.028	**0.003**
LnCRP	0.06	0.011	0.16	0.017	**0.03**	0.025	**0.01**
Pelvis BMD (g/cm^2)							
Age	0.07	0.020	0.15	0.0093	0.33	-0.0045	0.58
BMI	0.004	0.00032	0.98	0.013	0.19	0.028	**0.01**
LnPCB 180 (ng/g)[e]							
Left arm BMD (g/cm^2)							
BMI	0.01	0.0017	0.64	0.0065	**0.05**	0.012	**0.002**
LnCRP	0.07	0.0034	0.31	0.0065	0.06	0.011	**0.02**
Lipids	0.04	0.0026	0.50	0.0054	0.11	0.0082	**0.02**
Left leg BMD (g/cm^2)							
Age	0.07	0.019	**0.04**	0.013	0.07	0.0053	0.43
BMI	0.005	0.0079	0.28	0.016	**0.03**	0.025	**0.004**
LnCRP	0.04	0.011	0.11	0.016	**0.04**	0.022	**0.01**
Lipids	0.04	0.0092	0.24	0.014	**0.05**	0.019	**0.01**
Lumbar spine BMD (g/cm^2)							
BMI	0.05	0.0075	0.46	0.017	0.06	0.028	**0.01**
Antidiabetics	0.08	0.0084	0.70	0.028	0.59		
Pelvis BMD (g/cm^2)							
Age	0.08	0.022	0.17	0.011	0.33	-0.0025	0.80
BMI	0.02	0.0048	0.69	0.016	0.18	0.028	**0.03**
LnCRP	0.05	0.0088	0.45	0.015	0.19	0.025	0.07

All estimates were adjusted for the survey design and sample weights.

[a]Conditional effects are shown for significant and borderline significant interaction terms (p<.10).

[b]*P*-values in bold are statistically significant at p<.05.

[c]Multiple linear regression models were adjusted for age, BMI, lipids, race, PIR, survey cycle, bisphosphonates, and thiazide diuretics.
[d]Multiple linear regression models were adjusted for age, BMI, lipids, race, PIR, survey cycle, and cotinine.
[e]Multiple linear regression models were adjusted for age, BMI, lipids, race, PIR, and survey cycle.
[f]Beta for 1999–2000, beta for 2001–2002, and beta for 2003–2004 are shown.

APPENDIX B
TABLE XII

ASSOCIATIONS OF POPs WITH FSH AND LH BY POTENTIAL EFFECT MODIFIERS FOR 89 ELIGIBLE POSTMENOPAUSAL WOMEN, NHANES 1999–2004

		FSH (mIU/mL)[a]			FSH (mIU/mL)[b]			LH (mIU/mL)[a]			LH (mIU/mL)[b]		
POP	**Conditional effect**	**Interaction *p*-value[c]**	**Beta**	***p*-value[d]**	**Interaction *p*-value[c]**	**Beta**	***p*-value[d]**	**Interaction *p*-value[c]**	**Beta**	***p*-value[d]**	**Interaction *p*-value[c]**	**Beta**	***p*-value[d]**
Ln∑TEQ	Age	**0.03**											
(pg/g)	25th percentile		-7.1	0.19									
	50th percentile		-0.88	0.87									
	75th percentile		9.6	0.25									
	BMI	**0.03**			**0.04**								
	25th percentile		5.1	0.46		10.9	0.14						
	50th percentile		-2.7	0.62		3.9	0.53						
	75th percentile		-12.8	**0.05**		-5.3	0.47						
LnPCB	LnCRP							**0.02**			**0.05**		
138	25th percentile								2.3	0.34		2.7	0.26
(ng/g)	50th percentile								-1.2	0.52		-0.23	0.90
	75th percentile								-5.1	**0.03**		-3.5	0.17
	PIR	**0.001**			**0.002**								
	25th percentile		-6.3	0.06		-4.7	0.19						
	50th percentile		2.0	0.54		3.0	0.36						
	75th percentile		12.0	**0.01**		12.4	**0.01**						
LnPCB	LnCRP							**0.02**					
153	25th percentile								1.9	0.43			
(ng/g)	50th percentile								-1.6	0.39			
	75th percentile								-5.5	**0.02**			
	PIR	**0.001**			**0.002**								
	25th percentile		-6.4	0.05		-4.7	0.19						
	50th percentile		1.8	0.57		3.1	0.35						
	75th percentile		11.9	**0.02**		12.7	**0.01**						
	Cotinine	**0.02**											
	≤10 ng/mL		3.8	0.32									

APPENDIX B (continued)

POP	Conditional effect	FSH (mIU/mL)[a] Interaction *p*-value[c]	FSH (mIU/mL)[a] Beta	FSH (mIU/mL)[a] *p*-value[d]	FSH (mIU/mL)[b] Interaction *p*-value[c]	FSH (mIU/mL)[b] Beta	FSH (mIU/mL)[b] *p*-value[d]	LH (mIU/mL)[a] Interaction *p*-value[c]	LH (mIU/mL)[a] Beta	LH (mIU/mL)[a] *p*-value[d]	LH (mIU/mL)[b] Interaction *p*-value[c]	LH (mIU/mL)[b] Beta	LH (mIU/mL)[b] *p*-value[d]
	Cotinine >10 ng/mL		-13.1	**0.04**									
	Thyroid hormones										**0.03**		
	No											0.75	0.70
	Yes											-16.9	**0.03**
LnPCB	PIR	**0.01**			**0.02**								
180	25th percentile		-4.3	0.21		-2.0	0.58						
(ng/g)	50th percentile		1.1	0.73		3.0	0.39						
	75th percentile		9.5	0.07		10.8	**0.04**						
	Cotinine	**0.02**											
	≤10 ng/mL		5.2	0.18									
	>10 ng/mL		-13.8	**0.04**									
	Thyroid hormones							**0.03**			**0.02**		
	No								-1.2	0.55		0.46	0.82
	Yes								-19.8	**0.02**		-19.7	**0.02**
Ln∑PCBs	PIR	**0.001**			**0.002**								
(ng/g)	25th percentile		-6.9	0.09		-4.2	0.33						
	50th percentile		1.7	0.64		3.8	0.32						
	75th percentile		15.4	**0.008**		16.6	**0.005**						
	LnCRP							**0.05**					
	25th percentile								1.2	0.69			
	50th percentile								-2.1	0.34			
	75th percentile								-6.1	**0.04**			
Ln∑Non-	LnCRP							**0.02**					
dioxin-	25th percentile								1.9	0.50			
like PCBs	50th percentile								-2.1	0.33			
(ng/g)	75th percentile								-6.4	**0.02**			
	PIR	**0.002**			**0.004**								
	25th percentile		-6.7	0.09		-4.4	0.31						

APPENDIX B (continued)

POP	Conditional effect	FSH (mIU/mL)[a] Interaction *p*-value[c]	Beta	*p*-value[d]	FSH (mIU/mL)[b] Interaction *p*-value[c]	Beta	*p*-value[d]	LH (mIU/mL)[a] Interaction *p*-value[c]	Beta	*p*-value[d]	LH (mIU/mL)[b] Interaction *p*-value[c]	Beta	*p*-value[d]
	50th percentile		1.2	0.74		3.0	0.44						
	75th percentile		13.2	**0.02**		14.2	**0.02**						
	Cotinine	**0.03**											
	≤10 ng/mL		5.5	0.23									
	>10 ng/mL		-12.8	0.06									
	Thyroid hormones										**0.04**		
	No											0.62	0.79
	Yes											-20.1	**0.04**
Ln∑Mono-*ortho* PCBs (ng/g)	LnCRP							**0.03**					
	25th percentile								-3.4	0.14			
	50th percentile								0.98	0.76			
	75th percentile								-7.4	**0.007**			
	PIR	**0.003**			**0.005**								
	25th percentile		-10.6	**0.02**		-8.2	0.08						
	50th percentile		-3.7	0.33		-1.8	0.67						
	75th percentile		7.0	0.18		8.3	0.12						
	Race	**0.02**			**0.03**								
	Caucasian		7.1	0.32		7.9	0.27						
	African American		-8.0	0.25		-3.8	0.61						
	Other		-17.6	**0.02**		-14.9	**0.05**						
	Thyroid hormones	**0.04**			**0.01**			**0.005**			**0.01**		
	No		-2.8	0.48		2.0	0.63		-2.8	0.23		-0.23	0.92
	Yes		-41.7	**0.03**		-46.2	**0.01**		-34.5	**0.002**		-36.9	**0.001**
Ln∑Dioxin-like PCBs (ng/g)	BMI	**0.02**			**0.04**								
	25th percentile		8.4	0.16		10.5	0.08						
	50th percentile		0.40	0.92		3.5	0.42						
	75th percentile		-9.2	0.07		-4.9	0.37						
	PIR	**0.0012**			**0.0022**								

APPENDIX B (continued)

POP	Conditional effect	FSH (mIU/mL)[a] Interaction *p*-value[c]	Beta	*p*-value[d]	FSH (mIU/mL)[b] Interaction *p*-value[c]	Beta	*p*-value[d]	LH (mIU/mL)[a] Interaction *p*-value[c]	Beta	*p*-value[d]	LH (mIU/mL)[b] Interaction *p*-value[c]	Beta	*p*-value[d]
	25th percentile		-9.5	**0.04**		-6.6	0.18						
	50th percentile		-1.4	0.70		1.0	0.81						
	75th percentile		11.5	**0.03**		13.2	**0.02**						
Ln∑Cooke	LnCRP							**0.02**					
estrogenic	25th percentile								1.2	0.62			
PCBs	50th percentile								-2.4	0.20			
(ng/g)	75th percentile								-6.1	**0.01**			
	PIR	**0.0004**			**0.0009**								
	25th percentile		-7.0	**0.04**		-5.3	0.15						
	50th percentile		0.51	0.87		1.8	0.58						
	75th percentile		12.2	**0.02**		12.9	**0.01**						
	Race	**0.04**			**0.05**								
	Caucasian		10.9	0.12		11.4	0.12						
	African American		-9.9	0.23		-5.4	0.53						
	Other		-8.8	0.09		-6.8	0.20						
	Cotinine	**0.05**											
	≤10 ng/mL		3.1	0.43									
	>10 ng/mL		-11.5	0.07									
Ln∑Wolff	Thyroid hormones				**0.03**						**0.03**		
estrogenic	No					3.3	0.45					0.88	0.73
PCBs (ng/g)	Yes					-32.9	**0.04**					-20.1	**0.03**
Ln∑Cooke	BMI	**0.0071**			**0.02**			**0.03**					
anti-	25th percentile		12.1	0.08		13.4	0.05		1.8	0.65			
estrogenic	50th percentile		1.5	0.76		4.3	0.39		-3.1	0.26			
PCBs	75th percentile		-11.2	0.05		-6.6	0.29		-8.9	**0.009**			
(ng/g)	LnCRP	**0.03**						**0.02**			**0.05**		
	25th percentile		8.3	0.21					1.4	0.70		1.8	0.63

APPENDIX B (continued)

POP	Conditional effect	FSH (mIU/mL)[a] Interaction *p*-value[c]	Beta	*p*-value[d]	FSH (mIU/mL)[b] Interaction *p*-value[c]	Beta	*p*-value[d]	LH (mIU/mL)[a] Interaction *p*-value[c]	Beta	*p*-value[d]	LH (mIU/mL)[b] Interaction *p*-value[c]	Beta	*p*-value[d]
	50th percentile		0.44	0.93					-3.2	0.24		-2.4	0.42
	75th percentile		-9.0	0.12					-8.8	**0.008**		-7.4	**0.05**
	PIR	**0.001**			**0.002**								
	25th percentile		-10.6	**0.04**		-7.4	0.18						
	50th percentile		-0.25	0.95		2.3	0.63						
	75th percentile		16.2	**0.02**		17.7	**0.01**						
Ln∑Wolff	BMI	**0.03**											
anti-	25th percentile		8.1	0.18									
estrogenic	50th percentile		0.86	0.84									
PCBs	75th percentile		-7.8	0.13									
(ng/g)	PIR	**0.001**			**0.002**								
	25th percentile		-8.9	0.06		-5.8	0.24						
	50th percentile		-0.61	0.87		2.0	0.62						
	75th percentile		12.6	**0.03**		14.5	**0.01**						

[a]Models adjusted for age, BMI, and lnLipids.
[b]Models adjusted for age, BMI, lnLipids, and lnGGT.
[c]Conditional effects are shown for significant interaction terms ($p<0.05$).
[d]*P*-values in bold are statistically significant at $p \leq 0.05$.

APPENDIX C
TABLE XVIII

ASSOCIATIONS OF PCBS WITH FSH, ESTRADIOL, SHBG, SHBG-E2, AND FSH:E2 BY POTENTIAL EFFECT MODIFIERS FOR 77 ELIGIBLE POSTMENOPAUSAL WOMEN, GLFCS

		FSH (mIU/mL)[a]			LnEstradiol (pg/mL)[a]			SHBG (nmol/L)[a]			SHBG-E2[a]		
POP	**Conditional effect**	**Interaction *p*-value[b]**	**Beta**	***p*-value[c]**	**Interaction *p*-value[b]**	**Beta**	***p*-value[c]**	**Interaction *p*-value[b]**	**Beta**	***p*-value[c]**	**Interaction *p*-value[b]**	**Beta**	***p*-value[c]**
LnPCB	Antidiabetics	0.08											
163/138	No		-1.5	0.76									
(ng/g)	Yes		-86.4	0.08									
	BMI				0.098						**0.05**		
	25th percentile					0.001	0.99					1.7	0.32
	50th percentile					0.042	0.55					0.18	0.91
	75th percentile					0.095	0.21					-1.5	0.43
	Alcohol	0.09											
	<12 drinks/year		-11.4	0.09									
	≥12 drinks/year		2.8	0.63									
LnPCB	Antidiabetics	0.07											
132/153/	No		-3.4	0.50									
105	Yes		-85.3	0.06									
(ng/g)	BMI				0.09						**0.04**		
	25th percentile					0.004	0.96					2.5	0.20
	50th percentile					0.049	0.51					0.81	0.65
	75th percentile					0.11	0.18					-1.0	0.61
LnPCB	BMI				0.06						**0.05**		
180	25th percentile					0.049	0.58					3.9	0.07
(ng/g)	50th percentile					0.12	0.16					1.8	0.35
	75th percentile					0.20	**0.03**					-0.42	0.86
Ln∑PCBs	Antidiabetics	0.09											
(ng/g)	No		-9.1	0.19									
	Yes		-97.5	0.06									
	BMI				0.06						0.06		

APPENDIX C (continued)

		FSH (mIU/mL)[a]			LnEstradiol (pg/mL)[a]			SHBG (nmol/L)[a]			SHBG-E2[a]		
POP	**Conditional effect**	**Interaction *p*-value[b]**	**Beta**	***p*-value[c]**	**Interaction *p*-value[b]**	**Beta**	***p*-value[c]**	**Interaction *p*-value[b]**	**Beta**	***p*-value[c]**	**Interaction *p*-value[b]**	**Beta**	***p*-value[c]**
	25th percentile					0.011	0.92					3.2	0.21
	50th percentile					0.089	0.38					0.73	0.76
	75th percentile					0.19	0.10					-1.9	0.52
Ln∑Non-dioxin-like PCBs (ng/g)	Antidiabetics	0.08											
	No		-8.2	0.23									
	Yes		-111.7	0.06									
	BMI				**0.05**						**0.05**		
	25th percentile					0.009	0.93					3.1	0.22
	50th percentile					0.088	0.37					0.51	0.83
	75th percentile					0.19	0.10					-2.2	0.45
∑Mono-*ortho* PCBs (ng/g)[d]	Antidiabetics							**0.05**					
	No								-48.1	0.08			
	Yes								266.0	0.20			
	Thyroid hormones	0.07											
	No		-11.0	0.12									
	Yes		17.6	0.22									
	Age							**0.02**					
	25th percentile								-106.0	**0.03**			
	50th percentile								-51.1	0.17			
	75th percentile								11.6	0.77			
Ln∑Cooke estrogenic PCBs (ng/g)	Antidiabetics	0.095											
	No		-4.1	0.47									
	Yes		-76.0	0.07									
	BMI				0.08						**0.05**		
	25th percentile					-0.002	0.98					2.6	0.22

APPENDIX C (continued)

POP	Conditional effect	FSH (mIU/mL)[a] Interaction *p*-value[b]	FSH (mIU/mL)[a] Beta	FSH (mIU/mL)[a] *p*-value[c]	LnEstradiol (pg/mL)[a] Interaction *p*-value[b]	LnEstradiol (pg/mL)[a] Beta	LnEstradiol (pg/mL)[a] *p*-value[c]	SHBG (nmol/L)[a] Interaction *p*-value[b]	SHBG (nmol/L)[a] Beta	SHBG (nmol/L)[a] *p*-value[c]	SHBG-E2[a] Interaction *p*-value[b]	SHBG-E2[a] Beta	SHBG-E2[a] *p*-value[c]
	50th percentile					0.049	0.55					0.67	0.73
	75th percentile					0.12	0.19					-1.4	0.54
Ln∑Wolff	Antidiabetics	**0.05**											
estrogenic	No		-10.0	0.06									
PCBs	Yes		-76.2	**0.02**									
(ng/g)	BMI				**0.02**								
	25th percentile					0.085	0.29						
	50th percentile					0.17	**0.03**						
	75th percentile					0.27	**0.003**						
	Thyroid hormones	0.07											
	No		-15.1	**0.007**									
	Yes		6.5	0.56									
Ln∑Wolff	Antidiabetics							**0.04**					
anti-	No								-53.6	0.16			
estrogenic	Yes								210.4	0.08			
PCBs	Thyroid hormones	0.09											
(ng/g)	No		-13.3	0.06									
	Yes		13.3	0.35									

[a]Adjusted for age, BMI, and lipids.
[b]Conditional effects are shown for significant and borderline significant interaction terms ($p<.10$).
[c]*P*-values in bold are statistically significant at $p<.05$.
[d]∑Mono-*ortho* PCBs were dichotomized as <LOD and ≥LOD.

APPENDIX D

HUMAN SUBJECTS APPROVAL FOR AIM 1 AND AIM 2

UNIVERSITY OF ILLINOIS
AT CHICAGO

Office for the Protection of Research Subjects (OPRS)
Office of the Vice Chancellor for Research (MC 672)
203 Administrative Office Building
1737 West Polk Street
Chicago, Illinois 60612-7227

Notice of Determination of Human Subject Research

June 3, 2011

20110458-61053-1

20110458-61053-1

Anissa Therese Lambertino, MPH

Epidemiology and Biostatistics

1603 W Taylor St, M/C 923

Chicago, IL

Phone: (312) 413-3496 / Fax: (312) 996-7726

RE: **Protocol # 2011-0458**
Hormone Disruption by Organochlorines in Postmenopausal Women

Dear Ms. Lambertino:

☒The UIC Office for the Protection of Research Subjects received your "Determination of Whether an Activity Represents Human Subjects Research" application, and has determined that this activity **DOES NOT meet the definition of human subject research** as defined by 45 CFR 46.102(f).

You may conduct your activity without further submission to the IRB.

If this activity is used in conjunction with any other research involving human subjects or if it is modified in any way, it must be re-reviewed by OPRS staff.

APPENDIX D (continued)

☐ The UIC Office for the Protection of Research Subjects received your "Determination of Whether an Activity Represents Human Subjects Research" application, and has determined that this activity **DOES meet the definition of human subject research** as defined by 45 CFR 46.102(f).

You must submit either a Claim of Exemption or an Initial Review Application for IRB review. Your research cannot be conducted until written notice of an exemption determination or IRB approval has been granted.

For guidance on submitting your application, please refer to the guidance at:
http://tigger.uic.edu/depts/ovcr/research/protocolreview/irb/index.shtml

APPENDIX E

HUMAN SUBJECTS APPROVAL FOR AIM 3

UNIVERSITY OF ILLINOIS
AT CHICAGO

Office for the Protection of Research Subjects (OPRS)
Office of the Vice Chancellor for Research (MC 672)
203 Administrative Office Building
1737 West Polk Street
Chicago, Illinois 60612-7227

Approval Notice

Continuing Review

November 8, 2013

Mary Ellen Turyk, PhD

Epidemiology and Biostatistics

1603 W. Taylor St.

878-D S.P.H.P.I., M/C 923

Chicago, IL 60612-4394

Phone: (312) 355-4673 / Fax: (312) 996-0064

RE: Protocol # 2010-0003

"Diabetes and Persistent Organic Pollutants"

Dear Dr. Turyk:

Your Continuing Review was reviewed and approved by the Expedited review process on November 7, 2013. You may now continue your research.

Please note the following information about your approved research protocol:

Protocol Approval Period: December 6, 2013 - December 6, 2014

Approved Subject Enrollment #: 954 (600 enrolled to date, Closed to enrollment)

Additional Determinations for Research Involving Minors: These determinations have not been made for this study since it has not been approved for enrollment of minors.

Performance Sites: UIC, Wisconsin Division of Public Health

Sponsor: None

Research Protocol(s):

a) Diabetes and Persistent Organic Pollutants, Version 5, 5/21/2012

Recruitment Material(s):

a) N/A; Closed to Enrollment

Informed Consent(s):

a) N/A; Closed to Enrollment

HIPAA Authorization(s):

a) N/A; Closed to Enrollment

Your research meets the criteria for expedited review as defined in 45 CFR 46.110(b)(1) under the following specific category(ies):

(2) Collection of blood samples by finger stick, heel stick, ear stick, or venipuncture as follows:

(a) from healthy, nonpregnant adults who weigh at least 110 pounds. For these subjects, the amounts drawn may not exceed 550 ml in an 8 week period and collection may not occur more frequently than 2 times per week; or

(b) from other adults and children, considering the age, weight, and health of the subjects, the collection procedure, the amount of blood to be collected, and the frequency with which it will be collected. For these subjects, the amount drawn may not exceed the lesser of 50 ml or 3 ml per kg in an 8 week period and collection may not occur more frequently than 2 times per week.,

(5) Research involving materials (data, documents, records, or specimens) that have been collected, or will be collected solely for nonresearch purposes (such as medical treatment or diagnosis).,

(7) Research on individual or group characteristics or behavior (including but not limited to research on perception, cognition, motivation, identity, language, communication, cultural beliefs or practices and social behavior) or research employing survey, interview, oral history, focus group, program evaluation, human factors evaluation, or quality assurance methodologies.

Please note the Review History of this submission:

Receipt Date	Submission Type	Review Process	Review Date	Review Action
10/31/2013	Continuing Review	Expedited	11/07/2013	Approved

Please remember to:

→ Use your **research protocol number** (2010-0003) on any documents or correspondence with the IRB concerning your research protocol.

→ Review and comply with all requirements on the enclosure,

"UIC Investigator Responsibilities, Protection of Human Research Subjects"
(http://tigger.uic.edu/depts/ovcr/research/protocolreview/irb/policies/0924.pdf)

Please note that the UIC IRB has the prerogative and authority to ask further questions, seek additional information, require further modifications, or monitor the conduct of your research and the consent process.

Please be aware that if the scope of work in the grant/project changes, the protocol must be amended and approved by the UIC IRB before the initiation of the change.

We wish you the best as you conduct your research. If you have any questions or need further help, please contact OPRS at (312) 996-1711 or me at (312) 413-1835. Please send any correspondence about this protocol to OPRS at 203 AOB, M/C 672.

Sincerely,

Jonathan W. Leigh, MPH

IRB Coordinator, IRB # 1

Office for the Protection of Research Subjects

cc: Ronald C. Hershow, Epidemiology and Biostatistics, M/C 923

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PUBLICATIONS:
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