Persistent Organic Pollutants, Circulating Sex Hormones, and Coronary Heart Disease

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THESIS

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LIST OF ABBREVIATIONS

3α-ADG	3α-androstanediol glucuronide
3α-ADiol	3α-androstanediol
ADT	androgen deprivation therapy
AHA	American Heart Association
BMI	body mass index
CDC	Center for Disease Control
CHD	coronary heart disease
CI	confidence interval
CRP	c-reactive protein
CV	coefficient of variation
CVD	cardiovascular disease
CYP	cytochrome P-450
DDD	dichlorodiphenyldichloroethane
DDE	dichlorodiphenyldichloroethylene
DDT	dichlorodiphenyltrichloroethane
DHT	dihydrotestosterone
E2	estradiol
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
Freq.	frequency
Geomean	geometric mean
GGT	gamma-glutamyl transferase
GLSCF	Great Lakes sport-caught fish
HR	hazard ratio
HS	high school
MC	3-methylcholanthrene

LIST OF ABBREVIATIONS (CONTINUED)

MDL	method detection limit
MI	myocardial infarction
MICE	multiple imputations by chained equations
n-3 PUFA	omega-3 polyunsaturated fatty acid
NHANES	National Health and Nutrition Examination Survey
OC	Organochlorine
PAH	polyaromatic hydrocarbon
PB	phenobarbital
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzodioxin
PCDF	polychlorinated dibenzofuran
POP	persistent organic pollutant
PY	person-years
Rx	prescription
SHBG	steroid hormone binding globulin
T	testosterone
T4	thyroxine
TCDD	2,3,7,8-tetrachlorodibenzodioxin
TEQ	TCDD toxic equivalent
US	United States

SUMMARY

This study aimed to (1) explore longitudinal relationships between fish consumption, POPs, and CHD-related outcomes in the GLSCF Consumer Cohort, (2) explore relationships over time between POPs and endogenous steroid hormones in the GLSCF Consumer Cohort, and (3) explore cross-sectional relationships between POPs and endogenous steroid sex hormones in using data from the NHANES. The Great Lakes Fish Consumer Study is a 24-year longitudinal study based on a cohort established in 1992 by the Great Lakes Consortium, a collaboration of state health departments of states bordering the Great Lakes. Study subjects were selected from active Great Lakes charter boat captains, locally matched infrequent Great Lakes fish consumers, Wisconsin inland anglers, and spouses of participants.

Proportional hazards regression models were used to estimate longitudinal relationships between fish consumption, POPs, and CHD-related outcomes using data from the GLSCF Consumer Cohort. Likelihood-based mixed models were used to estimate associations of SHBG, testosterone and testosterone binding with DDE and PCB groupings over time using data from the GLSCF Consumer Cohort. Cross-sectional relationships between POPs and endogenous steroid sex hormones were explored using data from NHANES (1999-2002). The final analytic sample included 595 male participants. Linear regression models were used to estimate cross-sectional associations of PCB with circulating steroid sex hormones.

The results of the first study demonstrated a significantly increased risk of self-reported physician diagnosis of CHD with increasing serum concentrations of PCB congeners which are known phenobarbital-type (PB-type) inducers of metabolic cytochrome P450 enzymes of the 2A and 2B family. Specifically, after adjusting for known and suspected confounders, there was a

72% increase in risk of CHD for each doubling of serum concentrations of PB-type PCB congeners (p = 0.0294).

In the second study, PCBs were also associated with steroid sex hormones, which may potentially be a pathway through which PCBs increase risk of CHD. After excluding participants with liver disease, inverse associations of PCBs with SHBG (-16.5% for each doubling of PB-type PCBs, p=0.03) and SHBG-bound testosterone (-17.9%, p=0.02), paired with associations in the opposite direction for free testosterone (9.7%, p=0.07), are consistent with a PCB-induced inhibition of SHBG synthesis resulting in reduced binding of total testosterone to SHBG and leaving a larger proportion of testosterone circulating freely in the blood. We found a similar significant inverse association of DDE with SHBG-bound testosterone (-22.9%, p=0.03), but limited evidence for an impact of DDE on SHBG (-8%, p=0.14) and free testosterone (2.8%, p=0.45).

In the cross-sectional study using data from NHANES, PB-type PCBs were associated with reduced 5α-reductase activity in androgen metabolism, as measured by 3a-androstanediol glucuronide in men. Specifically, in the youngest age group, we found a 17% reduction in 3α-ADG for each 50% increase in PB-type PCBs (p=0.001) and this association became weaker with age, resulting in no observed association in the oldest age group. We also observed a 19% increase in total testosterone in only the oldest age group (p=0.046), and after standardizing to total testosterone, the inverse association of PB-type PCBs with 3α-ADG was consistent across all age groups, indicating an overall reduction in 5α-reductase activity with increasing PB-type PCBs. Furthermore, PB-type PCBs were associated with an 8% increase in SHBG across all age

groups (p=0.009), though this association was potentially mediated by the increase in total testosterone in the oldest age group.

Overall, results from this study provide evidence that PCBs, and particularly PCB congeners that are known PB-type inducers of CYP2A and CYP2B, may increase risk of CHD and the increase in risk of CHD may, at least in part, be a result of modified androgen homeostasis in males. The difference in results according to PCB grouping method highlights the importance of developing new methods for assessing the impact of multiple exposures to environmental pollutants in the presence of highly correlated exposure mixtures.

State public health agencies should continue to promote consumption of fatty fish for improved heart health, since fatty fish are an excellent source of omega 3 polyunsaturated fatty acids, but it is important to highlight in public fishing advisories that consuming fish from lakes known to be contaminated with PCBs may counteract the health benefits of fish consumption. Specifically, fish advisories should continue to suggest avoidance of large predatory fish of the Great Lakes, such as salmon, walleye, and trout, particularly from lakes with higher concentrations of PCBs, such as Lake Michigan, Lake Huron, and Lake Ontario.

1. INTRODUCTION

1.1. Fish Consumption and Coronary Heart Disease

Despite a drastic reduction in coronary heart disease (CHD) related mortality over the past 50 years, CHD remains the leading cause of death in the United States (1). Due to advances in pharmaceutical treatments and surgical interventions, fatal outcomes can be delayed or prevented, increasing the life expectancy of patients with advanced CHD. However, the monetary costs and psychological stress of these interventions can be burdensome to the patient, the patient's family, and to society as a whole (1). While treatment of CHD has become effective, prevention through promotion of healthy diet and lifestyle also plays an important role. One such preventative dietary intervention is regular consumption of fish. The American Heart Association (AHA) recommends consumption of at least two servings of fatty fish per week for the prevention of CHD (2). The cardioprotective effect of fish consumption has been consistently demonstrated in the literature and largely attributed to fatty fish being an excellent source of long-chain omega-3 polyunsaturated fatty acids (n-3 PUFAs) (3-11). Despite the observed protective effect of n-3 PUFAs from fish on risk of CHD, controlled trials of n-3 PUFA nutritional supplementation are inconsistent and do not demonstrate the same protective effect of n-3 PUFAs on CHD-related mortality seen in observation studies of fish consumption (12).

Since there is little evidence for a protective effect of nutritional supplementation of n-3 PUFAs or fish oils against CHD, consumption of fish continues to be recommended by clinicians and public health agencies as a preventative measure. However, all fish are not equivalently heart-healthy. Fish also tend to accumulate large quantities of environmental pollutants such as polychlorinated biphenyls (PCBs), dichlorodiphenyldichloroethylene (DDE), dioxins, furans, and methyl mercury. Many environmental pollutants have been associated with increased risk of

CHD and, since fish have generally been accepted as a healthy source of protein and n-3 PUFAs, it is important to weigh the risks and benefits of fish consumption and potentially modify dietary recommendations to prevent exposure to environmental pollutants. Fish of the North American Great Lakes are especially high in many of these pollutants due to a long history of pollution in the Great Lakes. With sport fishing being a popular recreational activity in the Great Lakes, we are presented with a potentially high-risk group, as Great Lakes anglers consume substantial quantities of contaminated fish (13).

1.2. Persistent Organic Pollutants and Coronary Heart Disease

Persistent organic pollutants (POPS) are chemicals that persist in the environment for long periods of time. Organochlorines are a class of highly lipophilic chemicals which tend to bind to organic matter and accumulate in adipose tissues. This property allows these chemicals to accumulate in organisms and magnify as they move up the food chain, ultimately ending up in humans through consumption of contaminated fish and other contaminated meat or dairy products. Once in humans, these chemicals are slowly eliminated from the body resulting in biologic half-lives of up to a decade.

Polychlorinated dibenzo dioxins, often referred to simply as ‘dioxins’, are a family of 75 compounds divided into 8 groups. Dioxins consist of two benzene rings with varying degrees of chlorination bound by two oxygen bridges at positions 1 and 6. The central ring, formed as a result of the two oxygen bridges, keeps the two benzene rings locked in a single plane (i.e. coplanar). They are formed as a byproduct of organochloride pesticide production, pulp and paper production, coal combustion, and industrial/municipal waste incineration. The latter two are understood to be the primary sources of dioxins in the environment.

Polychlorinated biphenyls (PCBs) are a family of 209 compounds, or congeners, very similar in structure to dioxins. PCBs consist of two benzene rings with varying degrees of chlorination bound by a single C-C bond at position 1. Steric hindrance between the two benzene rings prevents rotation of the molecule at the C-C bond between the two benzene rings, but with only one bond between the two, it is possible for the rings to lie in the same plane (coplanar) as well as in different planes (non-coplanar), depending on the position(s) of the chlorine substitutions on the benzene rings. It is for this reason that coplanar PCBs are often referred to as "dioxin-like" while non-coplanar PCBs are often referred to as "non-dioxin-like". Furthermore, because of the shared mechanism of action between "dioxin-like" PCBs and dioxins, a well-documented and validated method has been developed to calculate total dioxin and dioxin-like activity. This method applies a weight called the toxic equivalency factor (TEF) to each dioxin and dioxin-like PCB where the TEF is equal to the potency/affinity to the aryl hydrocarbon receptor in relation to the most toxic dioxin, 2,3,7,8-Tetrachlorodibenzodioxin (TCDD). The weighted TCDD equivalents are then summed as the total toxic equivalents (TEQ).

Unlike dioxins, PCBs were produced intentionally and widely used as lubricants and coolants/flame retardants in electrical capacitors and other electrical equipment. PCBs were first produced in the United States in the 1920's with peak production and use in the 1970's until production was banned in 1979 following conclusive evidence of negative health effects as a result of exposure to PCBs. There are no natural sources of PCBs and, although production was banned in 1979, PCBs produced prior to the ban still exist in the environment in part due to their strong stability and environmental persistence, but also due to current sources of PCB exposure, such as leakage from existing electrical capacitors produced before 1979. Thus, many congeners are still detectable in the serum of the general United States (US) population.

Dichlorodiphenyltrichloroethane (DDT) is an organochloride pesticide that was manufactured and used widely in the mid 1900's until its ban on use in the US in 1973. It is still manufactured and used in other nations specifically for the control of malaria. Dichlorodiphenyltrichloroethane consists of a central carbon substituted with two mono-chlorinated benzene rings and a trichloromethyl group. There are 3 isomers of DDT according to the positions of the chorines on the benzene rings. Other less persistent byproducts and metabolites of DDT are dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD), in which the trichloromethyl group is replaced with a dichloromethyl group or a monochloromethyl group, respectively. Despite being banned for over 40 years, DDT and its metabolites can still be detected in the environment and in the general human population.

Although the literature investigating associations of PCBs with cardiovascular disease is limited, positive associations have consistently been reported. Increased risk of cardiovascular disease (CVD) mortality has been demonstrated in two of three occupational cohorts of highly exposed workers (14-16). Studies of individuals living in proximity to large-scale environmental exposures have reported both increased risk of CVD mortality (17,18) and increased risk of hypertension (19). Similarly, studies involving two incidents of PCB contamination of rice oil reported increased risk of CVD mortality in individuals who consumed the contaminated rice oil compared to those who did not (20).

While these are examples of highly exposed individuals, PCBs at concentrations similar to the general US population have also been associated with increased risk of cardiovascular disease and hypertension. An ecologic study of hospital discharge in New York demonstrated increased odds of CHD and myocardial infarction (MI) discharge diagnosis among those living

near POPs contaminated areas (21). A cross-sectional relationship between serum PCBs and self-reported cardiovascular disease was reported among Akwesanse Mohawks (22) as well as the general US population in the National Health and Nutrition Examination Survey (NHANES) (23), but the latter study found this association only among females. Similarly, another cross-sectional study of Swedish elderly reported associations of PCBs with three different direct measures of atherosclerotic progression (24). Two prospective studies of elderly males (25) and females (26) found increased risk of incident myocardial infarction among the highest quartile of estimated dietary PCBs compared to the lowest, but only after adjusting for estimated dietary n-3 PUFA intake. While these few studies consistently report positive associations of PCBs and CHD-related outcomes, the results are inconsistent with respect to exposure and/or outcome classification as well as by sex and age. The literature on associations of DDE with CHD-related outcomes is sparse with little evidence suggesting increased risk of CHD with increasing levels of DDE.

Exposure to POPs has been associated with CHD risk factors. Several studies have demonstrated a cross-sectional association of DDE or PCBs with prevalent hypertension, but associations vary depending on how POPs mixtures are classified for analysis, adjustment for confounders, and age of the study sample (27-30). Furthermore, a longitudinal study of 973 individuals aged 50-65 in Belgium reported a significant association of self-reported incident hypertension with PCBs as well as total toxic equivalents (31). There is growing literature suggesting that increasing concentrations of serum DDE are associated with both prevalent (32-34) and incident diabetes (35,36), independent of body mass index (BMI), in populations exposed to background concentrations. There are studies that have reported associations between PCB exposure and BMI (37,38), but there is much controversy with regard to this topic since

there is large potential for reverse causality. Similarly, it has been shown that weight loss results in higher serum concentrations of POPs due to rapid release of POPs stored in adipose tissue (39,40). Researchers have also demonstrated associations of serum PCBs and organochlorine (OC) pesticides with serum cholesterol and with serum triglycerides (22,41). However, it is unclear whether this is a result of measurement error due to the strong lipophilicity of POPs, reverse causality or confounding by the strong association of diabetes with multiple exposure to DDT/PCBs.

It should be noted that the current literature, as well as major public health and environmental organizations such as the Environmental Protection Agency (EPA), the Center for Disease Control (CDC), and the US Food and Drug Administration (FDA), have concluded that consumption of large muscular fish is a major source of organic mercury. Exposure to organic mercury is understood to reduce the antioxidant capacity of the blood (42-45) resulting in platelet aggregation (46), vascular inflammation (44,45), and dyslipidemia (42), all of which are involved in atherosclerosis and increased risk of CHD-related outcomes (42).

1.3. The Role of Sex Hormones

There are many inconsistencies in results and exposure/outcome classification in studies exploring associations of POPs with sex hormones, resulting in limited evidence for causality in the presence of multiple exposures to environmental pollutants. More recent research in this field has focused on grouping POPs according to potency in a specific causal mechanism such as dioxin-like PCB congener groupings according to their potency in activating the aryl hydrocarbon receptor. Past methods have also focused on groupings of POPs according to their sex hormones disrupting activity (47,48) while more recent methods have focused on induction

of metabolic cytochrome P-450 (CYP) enzymes (49). The estrogenic or antiestrogenic effects of various groups of PCB congeners are largely a product of the CYP enzymes that are induced to metabolize PCBs, as many of the same enzymes are involved in steroid hormone metabolism. Thus, there is strong overlap between congeners grouped according to estrogenic properties and congeners grouped based on CYP induction. Specifically, the estrogenic and the anti-estrogenic PCB groups described by Wolff et al. (48) contains many of the same congeners as the phenobarbital (PB) type inducer and 3-methylcholanthrene (MC) type inducer PCB groups developed by Warner et al. (49), respectively.

Exposure to PCBs results in induction of CYPs to aid in metabolism and elimination of PCBs. Specifically, coplanar non-ortho substituted (dioxin-like) PCBs are strong MC-type inducers of metabolic enzymes CYP1A1, CYP1A2, and CYP1B1 while highly chlorinated non-coplanar ortho-substituted (non-dioxin-like) PCBs are strong PB-type inducers of metabolic enzymes CYP2B1, CYP2B2, CYP2A1, and CYP3A (50). Lower chlorinated mono-ortho-substituted PCBs can induce both MC-type and PB-type response and are referred to as mixed-type inducers (50). While these enzymes play a critical role in PCB metabolism and elimination, they are also involved in synthesis and metabolism of steroid sex hormones. Thus, induction of these enzymes has the potential to modify endogenous sex hormone profiles. Animal models have shown both estrogenic and anti-estrogenic PCB effects through abnormal development of sex organs (51,52) as well as sexual differentiation and behavior in the brain (53).

There is a wealth of literature supporting associations of sex hormones with CHD-related outcomes, but the results differ by sex and by endogenous vs exogenous sex hormones. Across all ages and multiple populations, researchers have observed an increased risk of CHD mortality in males compared to females (54), which has been largely attributed to differences in circulating

sex hormones. This is supported by a sharp increase in cardiovascular risk after females undergo menopause. Furthermore, results from the Women's Health Initiative demonstrated a cardioprotective effect of post-menopausal exogenous estrogen-only therapy among females who had undergone hysterectomy, but only in younger females who had recently undergone menopause (55,56). In contrast, there is evidence that post-menopausal estrogen+progestin therapy may increase risk of CHD (57,58). While the literature has consistently demonstrated a cardioprotective effect of estrogen in females, a cross-sectional study of post-menopausal elderly females demonstrated a 3-fold increase in odds of CHD among females in the highest quartile of total testosterone compared to the lowest quartile, but free testosterone was not associated with odds of CHD (59).

The cardioprotective effect of estrogen observed in females is not as well demonstrated in males. In fact, in contrast to females, testosterone appears to play a role in protection against CHD in males while elevated estrogen may be harmful. A 2011 systematic review and meta-analysis identified 19 eligible studies focused on associations of endogenous testosterone and risk of cardiovascular disease in healthy males. The meta-analysis found an inverse association of endogenous testosterone in healthy males, but only in studies of males over 70 years old (60). A second review and meta-analysis summarized results from 54 cross-sectional studies, 10 longitudinal studies, and 6 controlled trials (61). Among both cross-sectional studies and longitudinal studies, this meta-analysis reported an inverse association of total testosterone and a positive association of estradiol with cardiovascular disease prevalence or risk in males, regardless of cardiovascular disease ascertainment method.

The observed protective effect of circulating testosterone in males has sparked controversy in use of androgen deprivation therapy (ADT) for treatment of prostate cancer. In a

joint review by the American Heart Association, the American Cancer Society, and the American Urology Association, authors concluded that while there is evidence that androgen deprivation therapy increases risk of cardiovascular disease, it is unclear if the associations imply causality due to potential biases in the current literature (62). A pooled analysis of randomized controlled trials of androgen deprivation therapy in patients with prostate cancer demonstrated no difference in risk of CVD-mortality between patients receiving ADT and controls (63). However, randomized controlled trails are limited in their ability to detect differences in mortality risk due to the inherently short duration of clinical trials.

Though the literature is limited, there have been several cross-sectional studies suggesting that POPS may influence serum sex hormones. Over a total of 8 studies reporting associations of sex hormones with DDE, authors reported positive associations of DDE with free testosterone (64) and steroid hormone binding globulin (64,65) as well as negative associations of DDE and total testosterone (66-68), DHT (67), estradiol (69), and estrone sulfate (70). One study demonstrated a significant inverse association with a DDE isomer (71). Over a total of 7 studies reporting associations of sex hormones with any PCB congener or grouping, authors reported positive associations of PCBs with androstenedione (67), estrone (67), and steroid hormone-binding globulin (SHBG) (64,65) as well as negative associations of PCBs with total testosterone, SHBG, and SHBG-bound testosterone (70,72).

The mechanism through which sex hormones may modify cardiovascular risk is not clear, but it likely occurs through modification of lipid profiles and induction of a chronic state of systemic inflammation. Inflammation plays a major role in atherosclerotic progression. Specifically, atherosclerosis begins with oxidization of low-density lipoproteins, which prompt an immune response to endothelial cells of the blood vessel. White blood cells attack the

endothelial cells resulting in local inflammation, which eventually develops into atherosclerotic plaques resulting in narrowing and hardening of the artery and increased risk of ischemia. Many chronic diseases have been known to induce a chronic state of systemic inflammation which is suggested to more rapidly advance the atherosclerotic progression. Exhaustive resources have gone into studying chronic inflammation over the last few decades through biomarkers such as C-reactive protein (CRP), gamma-glutamyl transferase (GGT), adiponectin, fibrinogen, and interleukin-6. A pooled analysis of 54 long-term prospective studies including over 160,000 participants reported a strong association of CRP with coronary heart disease, ischemic stroke, and cardiovascular deaths (73). While this study focused primarily on CRP, there was strong correlation between CRP and other markers of chronic inflammation such as fibrinogen and interleukin-6. Furthermore, results from two well-established longitudinal studies, the Nurses' Health Study and the Health Professionals Follow-up Study, similarly suggest a significant association of CRP with risk of cardiovascular events (74).

Exposure to a class of POPs called dioxins is understood to induce a state of chronic systemic inflammation through activation of the aryl hydrocarbon receptor. Activation of the aryl hydrocarbon results in transcription of cytochrome P-450s, a class of enzymes which aid in metabolism and elimination of xenobiotics. Metabolism of dioxins and dioxin-like chemicals often results in proinflammatory radical oxygen species. The chemical structure of coplanar PCB congeners resembles dioxins, allowing activation of the aryl hydrocarbon receptor with varying degrees of potency depending on the congener (75). Like dioxins, metabolism of dioxin-like PCBs by cytochrome p450 into radical oxygen species results in a chronic state of systemic inflammation. A study of native Greenland Inuits demonstrated significant positive associations

of both OC pesticides and PCBs with two markers of chronic systemic inflammation, CRP and YKL-40 (76).

Similarly, research on dioxins and dioxin-like activity has demonstrated a consistent association with CHD-related outcomes. Male residents of the most contaminated area (Zone A) of Seveso, Italy following the 1976 industrial accident experienced a 5-fold increase in risk of death from chronic ischemic heart disease during the first 5 years following the incident (17,18). Similarly, a positive dose-response relationship was demonstrated between toxic equivalents of exposure to dioxins/furans and ischemic heart disease in a 40-year cohort of workers at an organochloride pesticide production plant in Hamburg, Germany (77,78). Finally, an international study of 36 cohorts from 12 countries which followed workers at organochloride herbicide production facilities for 40 years demonstrated a significant increase in risk of mortality from ischemic heart disease in workers exposed to TCDD or other highly chlorinated dioxins (14). While these studies did not explore the mechanism through which dioxins may have increased risk of cardiovascular mortality, the mechanism is likely related to an aryl hydrocarbon receptor-mediated state of systemic chronic inflammation and accelerated atherosclerotic progression.

1.4. Great Lakes Fish Contaminants and Fish Advisories

Since sport-caught fish are not regulated by US federal agencies such as the FDA or the EPA, fish advisories are released periodically to protect the public from consuming contaminated fish. Specifically, federal, state, and tribal agencies use epidemiologic evidence paired with biomonitoring data to make decisions on which species contain potentially harmful chemicals and how frequently those fish can be consumed in order to avoid adverse health effects. These

fish advisories protect consumers of sport-caught fish from ingesting highly contaminated species of fish acquired from highly contaminated waters. The advisories also provide information on preparing fish for consumption in order to reduce the amount of pollutants ingested, and often highlight the benefits of fish as a source of low-fat protein and nutrients such as omega 3 PUFAs.

Great Lakes sediments are contaminated with a wide variety of pollutants including synthetic organic chemicals (DDE and PCBs), fossil fuel components (polycyclic aromatic hydrocarbons - PAHs), and potentially toxic trace metals (arsenic, cadmium, lead, and mercury). The physical and chemical properties of many of these chemicals result in substantial bioaccumulation in large predatory fish through biomagnification up the food chain (79,80). Human consumption of contaminated Great Lakes fish is a source of exposure to these pollutants. The duration and quantity of Great Lakes fish consumption is reflected in higher body burdens of PCBs, polychlorinated dibenzo dioxins (PCDD) and furans (PCDF), persistent chlorinated pesticides, and mercury in studies of Great Lakes fish consumers (81-85). Results from a previous study of the Great Lakes fish consumer cohort demonstrate that on average, captains and their spouses who frequently consume of Great Lakes fish have significantly higher serum concentrations of PCBs than the participants of the 2003/2004 cycle of NHANES, a representative sample of the general US population (79) (Figure 1).

1.5. The Great Lakes Sport-Caught Fish Consumer Cohort

The Great Lakes Fish Consumer Study is based on a cohort established in 1992 by the Great Lakes Consortium, a collaboration of state health departments of states bordering the Great Lakes. Study subjects were selected from active Great Lakes charter boat captains licensed in

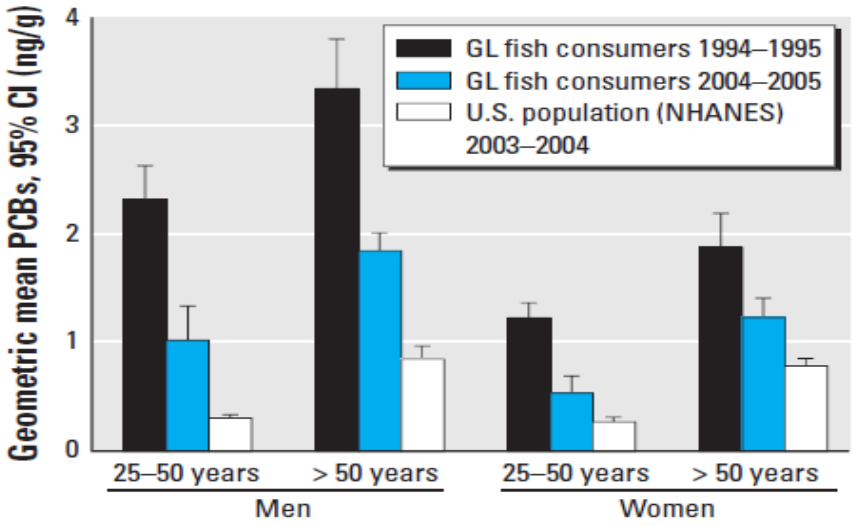

Figure 1. Sum of three PCB congeners in Great Lakes sport-fish consumers vs a representative sample of the general US population, adapted from Turyk et al. (140)

Wisconsin, Michigan, Ohio, Illinois, or Indiana. Spouses of charter boat captains were eligible if the couple had a child in 1970 or later. There was a total of 2,543 eligible participants between captains and spouses. Referents were selected from non-consumers of Great Lakes sport-caught fish (GLSCF) matched by city and telephone exchange. Eligibility criteria for referents included dietary history of no Great Lakes fish meals in the 12 months prior to the interview and fewer than 6 Great Lakes fish meals in the year prior to the interview. Spouses of referents were eligible if the couple had a child in 1970 or later. There was a total of 1,664 referents between matched non-consumers and their spouses. In addition to non-consumer referents, 180 inland anglers in Wisconsin and their spouses were invited. Telephone interviews were conducted in 1993 for GLSCF consumers and in 1994-1995 for referents to collect baseline demographic and GLSCF consumption information. Following the telephone survey, stratified random sampling

was used to select a subsample of 619 study subjects and referents for baseline blood serum biochemistry and POPs analysis.

Detailed health follow-up surveys were administered by phone or by mail in 1996, 2001, 2003, 2004, 2010, and 2017 to subsamples of 255, 207, 1788, 515, 598, and 234 respectively. Additional blood serum samples were collected for biochemistry, hormones, and POPs analysis in the 2001 and 2004 follow-up surveys. Since follow-up subsamples were selected with replacement, there were some individuals in which serum was collected in more than one follow-up. Serum POPs analysis was conducted at least once in a total of 945 study subjects/referents.

1.6. Significance

This study offers a rare opportunity to longitudinally investigate long-term exposure to environmental risk factors of CHD in a well-established cohort with up to 24 years of cardiovascular follow-up. Furthermore, the extensive list of measured biomarkers makes it possible to explore mechanisms of action, potentially offering additional evidence for causation in these associations. Finally, this study offers comparison of the results for hormone biomarkers to a much larger and more nationally representative sample of the general US population in a side-by-side comparison to a cross-sectional NHANES analysis.

Results from this study have the potential to influence Great Lakes fish consumption advisories, which provides information to Great Lakes charter boat captains that can help to reduce their exposure to harmful pollutants. While fish advisories are helpful to captains and their families in reducing exposure to harmful pollutants, there is also a much larger population of recreational anglers that may also benefit from the potential impact of this study.

Coronary heart disease is often difficult to study longitudinally since onset of symptoms or diagnosis do not occur until much later in life, but the uniquely long duration of this study allows for detection of differences in risk between the highly exposes and the less exposed. Furthermore, it can be very time-consuming and costly to measure environmental pollutants such as DDE and PCBs, as well as other important biomarkers such as cholesterol, triglycerides, sex hormones, and markers of inflammation. It requires extensive resources to collect serum samples and even more to have then analyzed in a certified laboratory by trained technicians using advanced analytic methods. Fortunately, we already have multiple measurements throughout the duration of the study of all biomarkers of interest apart from methyl mercury.

Cited Literature

1. Lloyd-Jones D, Adams RJ, Brown TM, et al. Heart disease and stroke statistics--2010 update: A report from the American Heart Association. *Circulation*. 2010;121(7):e46-e215.

2. American Heart Association. Fish and omega-3 fatty acids. http://www.heart.org/HEARTORG/GettingHealthy/NutritionCenter/HealthyEating/Fish-101_UCM_305986_Article.jsp#aha_recommendation. Updated 2014. Accessed 4/15, 2014.

3. He K, Song Y, Daviglus ML, et al. Accumulated evidence on fish consumption and coronary heart disease mortality: A meta-analysis of cohort studies. *Circulation*. 2004;109(22):2705-2711.

4. Burr ML, Gilbert J, Holliday Ra, et al. Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: Diet and reinfarction trial (DART). *The Lancet*. 1989;334(8666):757-761.

5. Zheng J, Huang T, Yu Y, Hu X, Yang B, Li D. Fish consumption and CHD mortality: An updated meta-analysis of seventeen cohort studies. *Public Health Nutr*. 2012;15(04):725-737.

6. Hu FB, Bronner L, Willett WC, et al. Fish and omega-3 fatty acid intake and risk of coronary heart disease in women. *JAMA*. 2002;287(14):1815-1821.

7. Oomen CM, Feskens EJ, Rasanen L, et al. Fish consumption and coronary heart disease mortality in Finland, Italy, and the Netherlands. *Am J Epidemiol*. 2000;151(10):999-1006.

8. Iso H, Kobayashi M, Ishihara J, et al. Intake of fish and n3 fatty acids and risk of coronary heart disease among Japanese: The Japan public health center-based (JPHC) study cohort I. *Circulation*. 2006;113(2):195-202.

9. Tomasallo C, Anderson H, Haughwout M, Imm P, Knobeloch L. Mortality among frequent consumers of Great Lakes sport fish. *Environ Res*. 2010;110(1):62-69.

10. Dyerberg J, Bang H, Stoffersen E, Moncada S, Vane J. Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis? *The Lancet*. 1978;312(8081):117-119.

11. Bang H, Dyerberg J, Nielsen A. Plasma lipid and lipoprotein pattern in Greenlandic west-coast Eskimos. *The Lancet*. 1971;297(7710):1143-1146.

12. Kwak SM, Myung S, Lee YJ, Seo HG, Korean Meta-analysis Study Group. Efficacy of omega-3 fatty acid supplements (eicosapentaenoic acid and docosahexaenoic acid) in the secondary prevention of cardiovascular Disease. A meta-analysis of randomized, double-blind, placebo-controlled Trials. Efficacy of omega-3 fatty acid supplements. *Arch Intern Med*. 2012;172(9):686-694.

13. Imm P, Knobeloch L, Anderson HA, Great Lakes Sport Fish Consortium. Fish consumption and advisory awareness in the Great Lakes basin. *Environ Health Perspect*. 2005:1325-1329.

14. Vena J, Boffetta P, Becher H, et al. Exposure to dioxin and nonneoplastic mortality in the expanded IARC international cohort study of phenoxy herbicide and chlorophenol production workers and sprayers. *Environ Health Perspect*. 1998;106 Suppl 2:645-653.

15. Gustavsson P, Hogstedt C. A cohort study of Swedish capacitor manufacturing workers exposed to polychlorinated biphenyls (PCBs). *Am J Ind Med*. 1997;32(3):234-239.

16. Prince MM, Hein MJ, Ruder AM, Waters MA, Laber PA, Whelan EA. Update: Cohort mortality study of workers highly exposed to polychlorinated biphenyls (PCBs) during the manufacture of electrical capacitors, 1940-1998. *Environ Health*. 2006;5:13.

17. Bertazzi PA, Bernucci I, Brambilla G, Consonni D, Pesatori AC. The Seveso studies on early and long-term effects of dioxin exposure: A review. *Environ Health Perspect*. 1998;106 Suppl 2:625-633.

18. Bertazzi PA, Consonni D, Bachetti S, et al. Health effects of dioxin exposure: A 20-year mortality study. *Am J Epidemiol*. 2001;153(11):1031-1044.

19. Goncharov A, Pavuk M, Foushee HR, Carpenter DO. Blood pressure in relation to concentrations of PCB congeners and chlorinated pesticides. *Environ Health Perspect*. 2011;119(3):319.

20. Li M, Tsai P, Chen P, Hsieh C, Guo YL, Rogan WJ. Mortality after exposure to polychlorinated biphenyls and dibenzofurans: 30 years after the “Yucheng accident”. *Environ Res*. 2013;120:71-75.

21. Sergeev AV, Carpenter DO. Hospitalization rates for coronary heart disease in relation to residence near areas contaminated with persistent organic pollutants and other pollutants. *Environ Health Perspect*. 2005;113(6):756-761.

22. Goncharov A, Haase RF, Santiago-Rivera A, et al. High serum PCBs are associated with elevation of serum lipids and cardiovascular disease in a native American population. *Environ Res*. 2008;106(2):226-239.

23. Ha MH, Lee DH, Jacobs DR. Association between serum concentrations of persistent organic pollutants and self-reported cardiovascular disease prevalence: Results from the National Health and Nutrition Examination Survey, 1999-2002. *Environ Health Perspect*. 2007;115(8):1204-1209.

24. Lind PM, van Bavel B, Salihovic S, Lind L. Circulating levels of persistent organic pollutants (POPs) and carotid atherosclerosis in the elderly. *Environ Health Perspect*. 2012;120(1):38-43.

25. Bergkvist C, Berglund M, Glynn A, Julin B, Wolk A, Åkesson A. Dietary exposure to polychlorinated biphenyls and risk of myocardial infarction in men—A population-based prospective cohort study. *Environ Int*. 2016;88:9-14.

26. Bergkvist C, Berglund M, Glynn A, Wolk A, Åkesson A. Dietary exposure to polychlorinated biphenyls and risk of myocardial infarction–A population-based prospective cohort study. *Int J Cardiol*. 2015.

27. Valera B, Ayotte P, Poirier P, Dewailly É. Associations between plasma persistent organic pollutant levels and blood pressure in Inuit adults from Nunavik. *Environ Int*. 2013;59:282-289.

28. Everett CJ, Mainous III AG, Frithsen IL, Player MS, Matheson EM. Association of polychlorinated biphenyls with hypertension in the 1999–2002 National Health and Nutrition Examination Survey. *Environ Res*. 2008;108(1):94-97.

29. Ha MH, Lee D, Son H, Park S, Jacobs D. Association between serum concentrations of persistent organic pollutants and prevalence of newly diagnosed hypertension: Results from the National Health and Nutrition Examination Survey 1999–2002. *J Hum Hypertens*. 2008;23(4):274-286.

30. Lind PM, Penell J, Salihovic S, van Bavel B, Lind L. Circulating levels of< i> p,< i> p'-DDE are related to prevalent hypertension in the elderly. *Environ Res*. 2014;129:27-31.

31. Van Larebeke N, Sioen I, Den Hond E, et al. Internal exposure to organochlorine pollutants and cadmium and self-reported health status: A prospective study. *Int J Hyg Environ Health*. 2014.

32. Lee DH, Lee IK, Song K, et al. A strong dose-response relation between serum concentrations of persistent organic pollutants and diabetes: Results from the National Health and examination survey 1999-2002. *Diabetes Care*. 2006;29(7):1638-1644.

33. Turyk M, Anderson HA, Knobeloch L, Imm P, Persky VW. Prevalence of diabetes and body burdens of polychlorinated biphenyls, polybrominated diphenyl ethers, and p, p′-diphenyldichloroethene in Great Lakes sport fish consumers. *Chemosphere*. 2009;75(5):674-679.

34. Silverstone AE, Rosenbaum PF, Weinstock RS, et al. Polychlorinated biphenyl (PCB) exposure and diabetes: Results from the Anniston community health survey. *Environ Health Perspect*. 2012;120(5).

35. Turyk M, Anderson H, Knobeloch L, Imm P, Persky V. Organochlorine exposure and incidence of diabetes in a cohort of Great Lakes sport fish consumers. *Environ Health Perspect*. 2009;117(7):1076-1082.

36. Wu H, Bertrand KA, Choi AL, et al. Persistent organic pollutants and type 2 diabetes: A prospective analysis in the Nurses' Health Study and meta-analysis. *Environ Health Perspect*. 2012;121(2):153-161.

37. Dirinck E, Jorens PG, Covaci A, et al. Obesity and persistent organic pollutants: Possible obesogenic effect of organochlorine pesticides and polychlorinated biphenyls. *Obesity*. 2011;19(4):709-714.

38. Lee D, Steffes MW, Sjödin A, Jones RS, Needham LL, Jacobs Jr DR. Low dose organochlorine pesticides and polychlorinated biphenyls predict obesity, dyslipidemia, and insulin resistance among people free of diabetes. *PloS one*. 2011;6(1):e15977.

39. Dirtu AC, Dirinck E, Malarvannan G, et al. Dynamics of organohalogenated contaminants in human serum from obese individuals during one year of weight loss treatment. *Environ Sci Technol*. 2013;47(21):12441-12449.

40. De Roos AJ, Ulrich CM, Sjodin A, McTiernan A. Adiposity, body composition, and weight change in relation to organochlorine pollutant plasma concentrations. *Journal of Exposure Science and Environmental Epidemiology*. 2012;22(6):617-624.

41. Arrebola JP, Ocana-Riola R, Arrebola-Moreno AL, et al. Associations of accumulated exposure to persistent organic pollutants with serum lipids and obesity in an adult cohort from southern Spain. *Environmental Pollution*. 2014;195:9-15.

42. Kobal AB, Horvat M, Prezelj M, et al. The impact of long-term past exposure to elemental mercury on antioxidative capacity and lipid peroxidation in mercury miners. *Journal of Trace Elements in Medicine and Biology*. 2004;17(4):261-274.

43. Kobal AB, Prezelj M, Horvat M, Krsnik M, Gibicar D, Osredkar J. Glutathione level after long-term occupational elemental mercury exposure. *Environ Res*. 2008;107(1):115-123.

44. Houston MC. The role of mercury and cadmium heavy metals in vascular disease, hypertension, coronary heart disease, and myocardial infarction. *Altern Ther Health Med*. 2007;13(2):S128-S133.

45. Houston MC. Role of mercury toxicity in hypertension, cardiovascular disease, and stroke. *The Journal of Clinical Hypertension*. 2011;13(8):621-627.

46. Kumar SV, Bhattacharya S. In vitro toxicity of mercury, cadmium, and arsenic to platelet aggregation: Influence of adenylate cyclase and phosphodiesterase activity. *In Vitro & Molecular Toxicology: A Journal of Basic and Applied Research*. 2000;13(2):137-144.

47. Cooke PS, Sato T, Buchanan DL, Robertson L, Hansen L. Disruption of steroid hormone signaling by PCBs. *PCBs Recent Advances in Environmental Toxicology and Health Effects*. 2001:257-263.

48. Wolff MS, Camann D, Gammon M, Stellman SD. Proposed PCB congener groupings for epidemiological studies. *Environ Health Perspect*. 1997;105(1):13-14.

49. Warner J, Osuch JR, Karmaus W, et al. Common classification schemes for PCB congeners and the gene expression of CYP17, CYP19, ESR1 and ESR2. *Sci Total Environ*. 2012;414:81-89.

50. Hansen LG. Stepping backward to improve assessment of PCB congener toxicities. *Environ Health Perspect*. 1998;106 Suppl 1:171-189.

51. Jansen HT, Cooke PS, Porcelli J, Liu T, Hansen LG. Estrogenic and antiestrogenic actions of PCBs in the female rat: In vitro and in vivo studies. *Reproductive Toxicology*. 1993;7(3):237-248.

52. Hany J, Lilienthal H, Sarasin A, et al. Developmental exposure of rats to a reconstituted PCB mixture or Aroclor 1254: Effects on organ weights, aromatase activity, sex hormone levels, and sweet preference behavior. *Toxicol Appl Pharmacol*. 1999;158(3):231-243.

53. Negri-Cesi P, Colciago A, Pravettoni A, Casati L, Conti L, Celotti F. Sexual differentiation of the rodent hypothalamus: Hormonal and environmental influences. *J Steroid Biochem Mol Biol*. 2008;109(3-5):294-299.

54. Lawlor DA, Ebrahim S, Smith GD. Trends in sex differences in mortality from heart disease: Sex is not same as gender, and theory was first proposed in 1950s, say authors. *BMJ*. 2002;324(7331):237-238.

55. Manson JE, Allison MA, Rossouw JE, et al. Estrogen therapy and coronary-artery calcification. *N Engl J Med*. 2007;356(25):2591-2602.

56. Rossouw JE, Prentice RL, Manson JE, et al. Postmenopausal hormone therapy and risk of cardiovascular disease by age and years since menopause. *JAMA*. 2007;297(13):1465-1477.

57. Toh S, Hernández-Díaz S, Logan R, Rossouw JE, Hernán MA. Coronary heart disease in postmenopausal recipients of estrogen plus progestin therapy: Does the increased risk ever disappear? A randomized trial. *Ann Intern Med*. 2010;152(4):211-217.

58. Hsia J, Langer RD, Manson JE, et al. Conjugated equine estrogens and coronary heart disease: The Women's Health Initiative. *Arch Intern Med*. 2006;166(3):357-365.

59. Patel SM, Ratcliffe SJ, Reilly MP, et al. Higher serum testosterone concentration in older women is associated with insulin resistance, metabolic syndrome, and cardiovascular disease. *The Journal of Clinical Endocrinology & Metabolism*. 2009;94(12):4776-4784.

60. Ruige JB, Mahmoud AM, De Bacquer D, Kaufman JM. Endogenous testosterone and cardiovascular disease in healthy men: A meta-analysis. *Heart*. 2011;97(11):870-875.

61. Corona G, Rastrelli G, Monami M, et al. Hypogonadism as a risk factor for cardiovascular mortality in men: A meta-analytic study. *Eur J Endocrinol*. 2011;165(5):687-701.

62. Levine GN, D'amico AV, Berger P, et al. Androgen-deprivation therapy in prostate cancer and cardiovascular risk: A science advisory from the American Heart Association, American Cancer Society, and American Urological Association: Endorsed by the American Society for Radiation Oncology. *CA: a cancer journal for clinicians*. 2010;60(3):194-201.

63. Nguyen PL, Je Y, Schutz FA, et al. Association of androgen deprivation therapy with cardiovascular death in patients with prostate cancer: A meta-analysis of randomized trials. *JAMA*. 2011;306(21):2359-2366.

64. Giwercman AH, Rignell-Hydbom A, Toft G, et al. Reproductive hormone levels in men exposed to persistent organohalogen pollutants: A study of Inuit and three European cohorts. *Environ Health Perspect*. 2006;114(9):1348-1353.

65. Haugen TB, Tefre T, Malm G, et al. Differences in serum levels of CB-153 and p, p′-DDE, and reproductive parameters between men living south and north in Norway. *Reproductive toxicology*. 2011;32(3):261-267.

66. Blanco-Munoz J, Lacasana M, Aguilar-Garduno C, et al. Effect of exposure to p,p'-DDE on male hormone profile in Mexican flower growers. *Occup Environ Med*. 2012;69(1):5-11.

67. Emeville E, Giton F, Giusti A, et al. Persistent organochlorine pollutants with endocrine activity and blood steroid hormone levels in middle-aged men. *PloS one*. 2013;8(6):e66460.

68. Freire C, Koifman RJ, Sarcinelli PN, Rosa ACS, Clapauch R, Koifman S. Long-term exposure to organochlorine pesticides and thyroid status in adults in a heavily contaminated area in Brazil. *Environ Res*. 2013;127:7-15.

69. Rylander L, Wallin E, Jönssson BA, Stridsberg M, Erfurth EM, Hagmar L. Associations between CB-153 and p, p′-DDE and hormone levels in serum in middle-aged and elderly men. *Chemosphere*. 2006;65(3):375-381.

70. Turyk ME, Anderson HA, Freels S, et al. Associations of organochlorines with endogenous hormones in male Great Lakes fish consumers and nonconsumers. *Environ Res*. 2006;102(3):299-307.

71. Asawasinsopon R, Prapamontol T, Prakobvitayakit O, Vaneesorn Y, Mangklabruks A, Hock B. Plasma levels of DDT and their association with reproductive hormones in adult men from northern Thailand. *Sci Total Environ*. 2006;355(1):98-105.

72. Persky V, Turyk M, Anderson HA, et al. The effects of PCB exposure and fish consumption on endogenous hormones. *Environ Health Perspect*. 2001;109(12):1275-1283.

73. Emerging Risk Factors Collaboration. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: An individual participant meta-analysis. *The Lancet*. 2010;375(9709):132-140.

74. Pai JK, Pischon T, Ma J, et al. Inflammatory markers and the risk of coronary heart disease in men and women. *N Engl J Med*. 2004;351(25):2599-2610.

75. Van den Berg M, Birnbaum LS, Denison M, et al. The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. *Toxicol Sci*. 2006;93(2):223-241.

76. Schæbel L, Bonefeld-Jørgensen EC, Vestergaard H, Andersen S. The influence of persistent organic pollutants in the traditional Inuit diet on markers of inflammation. *PloS one*. 2017;12(5):e0177781.

77. Flesch-Janys D. Elimination of polychlorinated dibenzo-p-dioxins and dibenzofurans in occupationally exposed persons. *Journal of Toxicology and Environmental Health Part A*. 1996;47(4):363-378.

78. Flesch-Janys D, Berger J, Gurn P, et al. Exposure to polychlorinated dioxins and furans (PCDD/F) and mortality in a cohort of workers from a herbicide-producing plant in hamburg, federal republic of Germany. *Am J Epidemiol*. 1995;142(11):1165-1175.

79. Turyk ME, Bhavsar SP, Bowerman W, et al. Risks and benefits of consumption of Great Lakes fish. *Environ Health Perspect*. 2012;120(1):11.

80. Adriaens P, Batterman S, Blum J, Hayes K, Meyers P, Weber W. Great Lakes sediment: Contamination, toxicity and beneficial. *Re-Use, SNRE*. 2002;37.

81. Cole DC, Sheeshka J, Murkin EJ, et al. Dietary intakes and plasma organochlorine contaminant levels among Great Lakes fish eaters. *Archives of Environmental Health: An International Journal*. 2002;57(5):496-509.

82. Falk C, Hanrahan L, Anderson HA, et al. Body burden levels of dioxin, furans, and PCBs among frequent consumers of Great Lakes sport fish. *Environ Res*. 1999;80(2):S19-S25.

83. Fitzgerald EF, Deres DA, Hwang S, et al. Local fish consumption and serum PCB concentrations among Mohawk men at Akwesasne. *Environ Res*. 1999;80(2):S97-S103.

84. Hanrahan LP, Falk C, Anderson HA, et al. Serum PCB and DDE levels of frequent gGreat Lakes sport fish consumers—a first look. *Environ Res*. 1999;80(2):S26-S37.

85. Kosatsky T, Przybysz R, Armstrong B. Mercury exposure in Montrealers who eat St. Lawrence river sportfish. *Environ Res*. 2000;84(1):36-43.

86. Turyk ME, Bhavsar SP, Bowerman W, et al. Risks and benefits of consumption of Great Lakes fish. *Environ Health Perspect*. 2012;120(1):11.

2. PURPOSE OF THE STUDY

2.1. Overall Goal

Our overall goal is to understand risks and benefits of GLSCF consumption with respect to coronary heart disease CHD while considering both potentially harmful environmental contaminants and heart-healthy n-3 PUFAs.

2.2. Aims and Objectives

To meet the overall goal of this study and speak to the larger question of risks and benefits of fish consumption, there were three specific aims to be addressed, each with its own set of objectives to be met. Upon completing these aims and objectives, we will better understand the risk associated with consumption of fish contaminated with POPs. This information can be used to guide state and local fish advisories in areas containing bodies of water with known contamination. We will also better understand potential mechanisms involved in any increased progression of CHD, which will aid in building evidence toward a causal relationship. Finally, with comparison to nationally representative NHANES data, our results will be generalizable to populations outside of the GLSCF consumer cohort.

2.2.1 Aim 1

The first aim was to explore longitudinal relationships between fish consumption, POPs, and CHD-related outcomes in the GLSCF Consumer Cohort. To address this aim, the following objectives were met:

1. Extend the longitudinal investigation of the GLSCF consumer cohort
2. Impute missing data on serum PCBs and other predictors of CHD-related outcomes using fully conditional specification of multiple imputations through chained equations

3. Estimate longitudinal associations of serum PCB concentrations with time to self-reported physician diagnosis of incident CHD-related outcomes using a series of proportional hazards regression models
4. Explore the roles of comorbid hypertension, comorbid diabetes, and serum lipids in the association of PCBs with CHD-related outcomes
5. Explore the role of Great Lakes fish consumption in the association of PCBs with CHD-related outcomes

2.2.2 Aim 2

The second aim was to explore relationships between POPs and endogenous steroid sex hormones over time in the Great Lakes Sport-Caught Fish Consumer Cohort

1. Impute missing data on serum PCBs and other predictors of circulating sex hormones using fully conditional specification of multiple imputations through chained equations
2. Estimate cross-sectional associations of serum PCB concentrations with circulating steroid sex hormones over time using linear mixed models

2.2.3 Aim 3

The third aim was to explore cross-sectional relationships between POPs and endogenous steroid sex hormones in using data from NHANES

1. Impute missing data on circulating sex hormones, serum PCBs, and other predictors of circulating sex hormones using fully conditional specification of multiple imputations through chained equations
2. Estimate cross-sectional associations of serum PCB concentrations with circulating steroid sex hormones using ordinary least squares linear regression models

3. LONGITUDINAL ASSOCIATIONS OF PCBS AND DDE WITH INCIDENT CHD-RELATED OUTCOMES

3.1. Introduction

Despite a drastic reduction in CHD mortality over the past 50 years, CHD remains the leading cause of death in the United States (1). Even among individuals with advanced CHD, fatal outcomes are now often prevented due to advances in pharmaceutical and surgical interventions, but the monetary costs and psychological stress of these interventions can be burdensome to individuals and to society as a whole (1). While treatment of CHD has become very effective, primary prevention through promotion of healthy diet and lifestyle also plays an important role. One such preventative dietary intervention is the consumption of fish. The American Heart Association recommends consumption of at least two servings of fatty fish per week for the prevention of CHD (2). The protective effect of fish consumption has been consistently demonstrated in the literature and has largely been attributed to fatty fish being an excellent source of long-chain n-3 PUFAs (3-6). Despite the observed protective effect of fish on risk of CHD, controlled trials of nutritional n-3 PUFA supplementation are inconsistent and do not demonstrate a protective effect of PUFAs (7).

Since nutritional supplementation of n-3 PUFAs is not effective in protection against CHD, fish consumption continues to be recommended by clinicians and public health professionals as a preventative measure. However, all fish are not equivalently heart-healthy. Some fish tend to accumulate environmental pollutants such as PCBs, DDT, and methyl mercury, all of which have been associated with increased risk of CHD or risk factors for CHD. While fish have generally been accepted as a healthy source of protein and n-3 PUFAs, it is important to weigh the risks and benefits of fish consumption and potentially modify dietary

recommendations to prevent exposure to environmental pollutants. Fish of the North American Great Lakes are especially high in many of these pollutants due to a long history of pollution in this region (8). With sport fishing being a popular recreational activity in the Great Lakes, we are presented with a potentially high-risk group, as Great Lakes anglers consume substantial quantities of contaminated fish (9).

Organochlorines are highly lipophilic and tend to bind to and accumulate in adipose tissue. Many organochlorines are eliminated very slowly compared to other xenobiotics resulting in biologic half-lives of up to a decade (10). This allows pollutants to accumulate and magnify as they move up the food chain, ultimately ending up in humans through consumption of contaminated fish, as well as contaminated meat or dairy products.

Dichlorodiphenyltrichloroethane is an organochlorine pesticide with widespread use for insect control until its use was banned in the United States in 1973. Due to its environmental persistence, DDT and its metabolites DDE and DDD still exist in the environment, the food chain, and in measurable quantities in the serum of the general US population. There is little evidence that DDE is associated with CHD, but there is a wealth of literature suggesting that higher serum DDE may increase risk of diabetes (11-15), a strong risk factor for CHD.

PCBs are a family of organochlorine compounds that were widely used in the US as coolants/flame retardants in electrical capacitors and other electrical equipment. PCBs were first produced in the United States in the 1920’s as mixtures of different congeners called Aroclors. Production and use peaked in the 1970’s until production was banned in 1979. Like DDE, PCBs produced prior to their ban still exist in the environment as a result of their environmental persistence and many congeners are still measurable in the general US population.

Although the literature is limited in studies investigating associations of PCBs with CHD, a positive association has consistently been reported. Increased risk of CHD mortality has been demonstrated in two of three occupational cohorts of highly exposed workers (16-18). Studies of individuals living in proximity to large-scale environmental exposures have reported both increased risk of CHD mortality (19,20) and increased risk of hypertension (21). Similarly, studies investigating two incidents in which rice oil was contaminated with PCBs and furans reported increased risk of CHD mortality in individuals who consumed contaminated rice oil (22). While these are examples of highly exposed individuals, PCBs at concentrations more similar to those expected in frequent consumers of Great Lakes sport-caught fish have also been associated with increased risk of CHD-related outcomes (23-26) as well as major risk factors for CHD such as hypertension (27-31), diabetes (32,33), and high cholesterol (34-36). However, these results often differ in methods used for exposure and/or outcome classification as well as by sex and age.

The biological mechanisms through which PCBs may increase risk of CHD differs by congener, as the mechanism depends on the structure of the congener and the group of enzymes which metabolize them. Coplanar PCBs have strong dioxin-like characteristics which allow them to bind to and activate the aryl hydrocarbon receptor (AhR), though some mono-ortho congeners can also activate the AhR with lesser potency. Activation of the AhR upregulates synthesis of CYP1A, and CYP1B, which then breaks down PCBs into metabolites containing highly reactive oxygen species. These highly reactive oxygen species circulate through the blood, increasing oxidative stress and causing damage to the basal cell layer of blood vessels, which results in the release of inflammatory cytokines and development of atherosclerotic plaques on the lining of

the blood vessels. Accelerated atherosclerosis eventually results in decreased blood flow to surrounding tissues, and can be life threatening, especially in the arteries of the heart.

While AhR activation is thought to be the primary pathway for dioxin-like PCBs, induction of metabolic cytochrome p-450 enzymes likely plays a role in the toxic effects all types of PCBs. Coplanar non-ortho substituted (dioxin-like) PCBs are understood to illicit an MC-type induction of CYP1A1, CYP1A2, and CYP1B1 while highly chlorinated non-coplanar ortho-substituted (non-dioxin-like) PCBs illicit a PB-type induction of CYP2B1, CYP2B2, CYP2A1, and CYP3A (37). Lower chlorinated mono-ortho-substituted PCBs are mixed-type inducers, which illicit both MC-type and PB-type responses (37). While these enzymes play a critical role in metabolism of xenobiotics such as PCBs, they are also involved in synthesis and metabolism of steroid sex hormones. Thus, induction of these enzymes has the potential to modify endogenous sex hormone profiles. Animal models have shown both estrogenic and anti-estrogenic PCB effects through abnormal development of sex organs (38,39) as well as sexual differentiation and behavior in the brain (40).

The purpose of this study is to investigate longitudinal associations of PCBs and DDE with incident diagnosis of CHD and related outcomes in a cohort of Great Lakes sport-caught fish consumers.

3.2. Methods

3.2.1 Cohort Background

The Great Lakes Fish Consumption Study was founded in 1992 by the Great Lakes Consortium. The original cohort consisted of 4397 participants at baseline including Great Lakes charter boat captains licensed in WI, IL, IN, MI, or OH, inland WI anglers, and infrequent consumers of Great Lakes sport-caught fish (referents) recruited from the same geographic areas

through random digit dialing. Spouses of participants were also included if the couple had a child in 1970 or later. Detailed description of participant selection is described elsewhere (41,42).

All 4397 participants completed a telephone survey at baseline addressing basic demographic information and fish consumption habits. A stratified sample of 619 participants was selected to donate blood samples for biomarker analysis in 1994-1995 (42). Of those 619, 225 completed a comprehensive health follow-up questionnaire in 1996, and 209 donated a second blood sample between 2001 and 2003. A cohort-wide comprehensive health follow-up questionnaire was completed by 1,788 of the original 4397 participants in 2003. Of those 1,788 who participated in the 2003 follow-up study, 515 donated a blood sample in 2004 while 598 and 234 completed comprehensive health follow-up questionnaires in 2010 and 2017, respectively. Supplementary health follow-up questionnaires were additionally administered at each of blood draws. Participants were included in this study if they donated at least 1 blood sample in 1994-1995, 2001-2003, or 2004, and they participated in at least 1 comprehensive health follow-up survey in 1996, 2003, 2010, or 2017. A detailed outline of the study can be seen in Figure 2.

3.2.2 Exposure Ascertainment

Sera were analyzed for DDE and 89 PCB congeners using electron capture capillary column gas chromatography. In 1994-1995, analysis efforts were split between the Wisconsin State Laboratory of Hygiene and the Michigan Department of Public Health Laboratory, while the latter two analyses were conducted entirely in Wisconsin.

Due to slight differences in analytic technique and instrumentation between laboratories and over time, only 18 PCB congeners represented by 13 unique peaks could be consistently quantified (74, 99, 118, 146, 180, 194, 201, 206, 132/153, 138/163, 170/190, 182/187, and 196/203). Analytes below the method detection limit (MDL) were imputed using censored

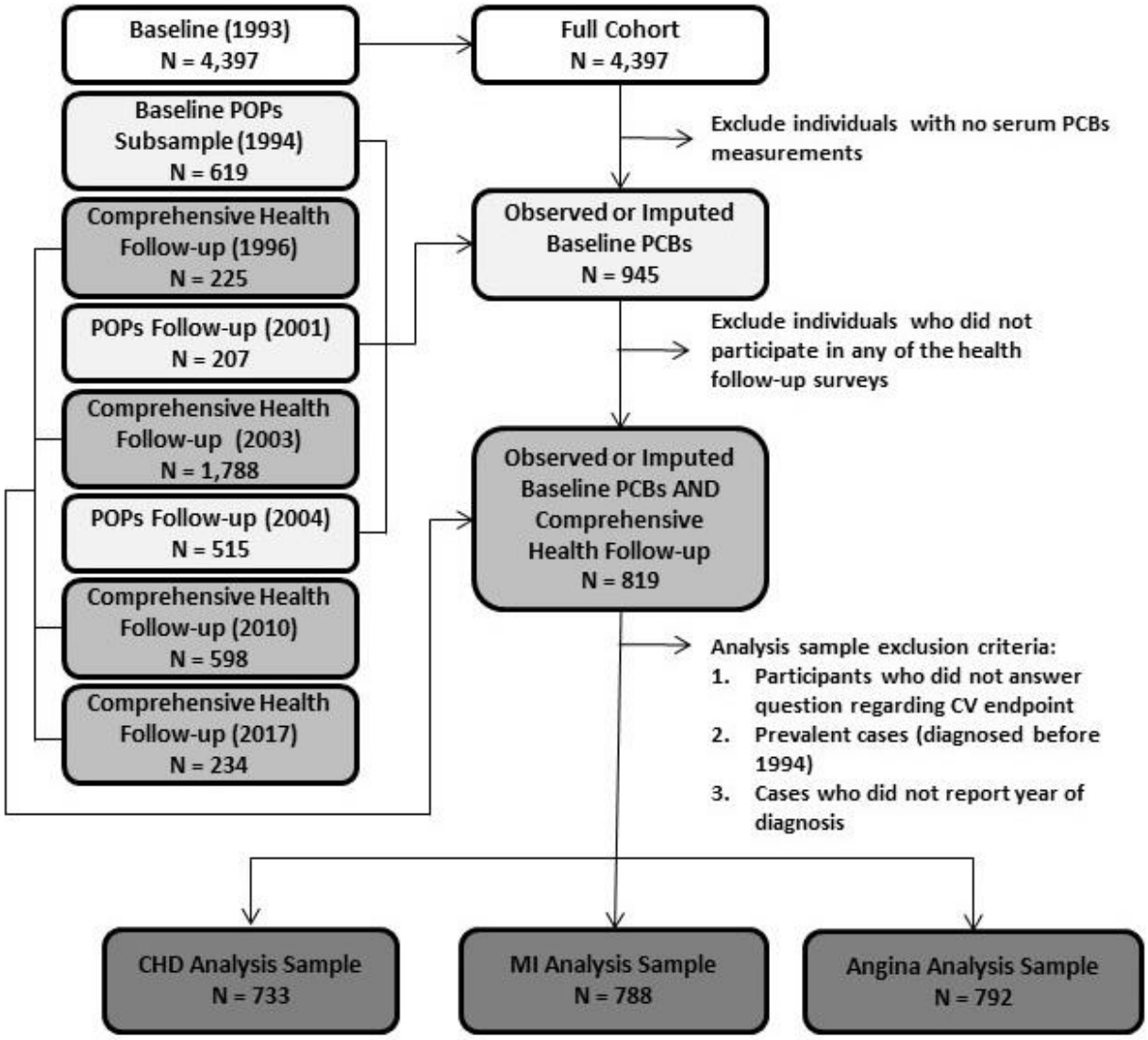

Figure 2. Study timeline and analytic sample inclusion criteria

maximum likelihood estimation (43) in R (44). Briefly, observations below the MDL were randomly assigned values between 0 and the respective MDL following a log-linear distribution of observations above the limit. For statistical analyses, PCB congeners were summed over all congeners and grouped according to their proposed metabolic pathways (45) for the following four PCB groupings: (1) Total PCBs, (2) MC-type PCBs, (3) PB-type PCBs, and (4) Mixed-type PCBs. A list of measured congeners and their associated grouping is presented in Table I.

3.2.3 Outcome Definitions and Ascertainment

Outcomes of interest in this study include CHD, myocardial infarction (MI), and angina pectoris. History of CHD-related outcomes was a self-reported physician diagnosis from comprehensive health follow-up questionnaires sent in 1996, 2001, 2003, 2010 and 2017 apart from history of CHD, which was not reported in 1996. Incident cases are defined as (1) having answered 'yes' to the question regarding physician diagnosis of CHD, MI, and angina, (2) having reported a year of diagnosis, and (3) must not have been diagnosed prior to baseline

TABLE I. PCB GROUPINGS AND INCLUDED PCB CONGENERS [a]

PCB Grouping	PCB Congeners Included [b]
Total PCBs	PCBs: 74, 99, 118, 132/153, 138/163, 146, 170/190, 180, 182/187, 194, 196/203, 201, 206
MC-type Inducer PCBs (45)	PCBs: 74, 118 (66, 70, 77, 105, 107, 110, 126, 156, 167)
Mixed-type Inducer PCBs (45)	PCBs: 99, 138, 170 (128, 157, 158, 167, 171, 177)
PB-type Inducer PCBs (45)	99, 151, 132/153, 180, 182/187, 187, 194, 196/203, 199, 201, 206 (17, 31, 44, 49, 52, 82, 87, 91, 92, 95, 97, 101, 110, 136, 141, 149, 151, 174, 177, 179, 183, 193, 195, 199, 205)

[a] PCB = Polychlorinated Biphenyl, MC = 3-methyl-cholanthrene, PB = phenobarbital

[b] PCB congeners in parentheses are included in the method for PCB grouping, but were not measured in any blood biomarker follow-up study

(1994). For patients who participated in multiple comprehensive health follow-up surveys, the first reported diagnosis was used. For patients who reported a diagnosis, but who failed to report a year of diagnosis, missing year of diagnosis was imputed as the current survey year if they had previously reported no diagnosis at a previous follow-up. Follow-up time is defined as time from baseline exposure ascertainment to either time of disease diagnosis or time of last follow-up (censorship date).

3.2.4 Covariate Definitions and Ascertainment

Demographic characteristics such as age, sex, body mass index (BMI), and education were reported at baseline. Race/ethnicity was omitted as a covariate since 99% of the study sample was non-Hispanic white. Income was excluded from the analysis due to a high proportion of missing values, likely missing not at random. Body mass index and smoking status were also reported at each comprehensive health follow-up questionnaire and each blood draw supplementary questionnaire. Smoking status was not reported at baseline, but smoking status at baseline was reported retrospectively in the 2010 comprehensive health questionnaire. Frequency of Great Lakes fish consumption and of any fish consumption were reported at baseline and at all follow-up questionnaires. Serum cholesterol and triglycerides were measured using samples collected from blood draws in 1996, 2001, and 2004. Total lipids were calculated using the following equation (46):

$$\text{Equation 1: } \text{Total Lipids} = (2.27 * \text{Total Cholesterol}) + \text{Triglycerides} + 62.3$$

3.2.5 Statistical Analysis

Due to multiple stratified sampling for biomarker analysis, only 14% of participants have complete records for POPs measurements at all 3 blood draws. Missing values for all POPs and covariates were imputed using the multiple imputations by chained equations (MICE) package in R 3.2.0 (R Foundation for Statistical Computing, Vienna, Austria). Details on the imputation method can be seen in Appendix A.

Proportional hazards regression was used to estimate longitudinal associations of total PCBs, MC-type PCBs, PB-type PCBs, mixed-type PCBs, and DDE with time to diagnosis of CHD, MI, and angina, using PROC PHREG in combination with proc MIANALYZE in SAS 9.4 (SAS Institute Inc., Cary, NC). All PCBs were lipid-standardized and natural log transformed. The final models included sex (male/female), age (continuous), age^2 (continuous), BMI (continuous, time-varying), BMI^2 (continuous, time-varying), smoking status (never/former/current), log-transformed serum lipids (continuous, time-varying), Great Lakes fish meals (continuous, time-varying) , Great Lakes fish meals^2 (continuous, time-varying), nearest Great Lake to residence, interactions between nearest Great Lake and Great Lakes fish meals, family history of CHD (yes/no), family history of heart attack (yes/no), and family history of diabetes (yes/no). BMI, serum lipids, and Great Lake fish meals were allowed to vary over time using linear interpolation between observed values while smoking used last observed time carried forward. The inclusion of both lipid standardization and lipid adjustment is recommended as best practice in a simulation and applied human exposure biomarker study (47). Proportional hazards were assumed if the estimate for the interaction between follow-up time and exposure was not statistically significant at alpha=0.05. Interaction terms for PCB groupings with sex, age, BMI, lipids, smoking, and Great Lakes fish meals were added individually and included in the

final model if the respective estimates were significant at alpha=0.05. Covariates were excluded from the final models if they did not predict outcome with a p-value of ≤0.4 (48). Time-dependent hypertension and diabetes were not included in the saturated models due to potential for mediation of the main associations but were added separately to assess the role of these variables in the main associations. Similarly, time-dependent total lipid standardization and adjustment were removed from the final model to assess the role of total lipids in the main associations. This study was approved by the institutional review boards (IRB) for human studies at University of Illinois in Chicago and at the University of Wisconsin Madison.

3.3. Results

There were 819 participants in the overall study sample at baseline (Figure 2), which consisted of primarily non-Hispanic white (98%) males (66%) between the ages of 40 and 60 years (69%). At baseline, most subjects were overweight or obese (68%), non-smokers (62%), with at least a high school diploma or equivalent (64%). On average, most participants at baseline consumed fish at least once every two weeks (58%). However, consumption of fish from the Great Lakes was less common with only 41% consuming fish at least once every two weeks. Univariate descriptive statistics are presented in Table II. All missing data on exposure and covariates were imputed.

Follow-up time was calculated as time from baseline PCB measurement to either time of event or time of last comprehensive health follow-up (years) and total follow-up time was calculated as the sum of follow-up time for all participants (person-years) while incidence rate was calculated as the cumulative incidence per 1000 person-years (PY). After excluding prevalent diagnoses and participants missing data on outcome, we observed 71 CHD diagnoses

over 11,518 person-years of follow-up (6.2 per 1000 PY) with median follow-up time of 15.6 years, 41 MI diagnoses over 12,030 person-years of follow-up (3.4 per 1000 PY) with median follow-up time of 15.5 years, and 30 angina diagnoses over 12,170 person-years of follow-up (2.5 per 1000 PY) with median follow-up time of 15.6 years (Table III).

Demographic and baseline factors associated with increased incidence of CHD-related endpoints throughout the study include sex (male), increasing age, increasing BMI, comorbid hypertension, comorbid diabetes, total lipids, and family history of CHD (Table II). Factors associated with elevated DDE and PCBs include sex (male), increasing age, increasing BMI, comorbid hypertension, comorbid diabetes, and Great Lakes fish consumption (Table IV). In the crude models, a doubling of total PCBs, PB-type PCBs, and mixed-type PCBs were associated with a 74% increase (95% CI: 1.17-2.56; p = 0.0058), 78% increase (95% CI: 1.20-2.65; p=0.0045), and 74% increase (95% CI: 1.12-2.39; p=0.0107) in likelihood of reporting diagnosis of CHD, respectively, while MC-type PCBs were not associated with risk of CHD. PCBs were not associated with risk of MI nor angina, regardless of grouping method (Table V).

After adjusting for known and suspected confounders (Model 1), CHD hazard ratio estimates for a 2-fold increase in PCBs were slightly attenuated as compared to the crude model for total PCBs (HR = 1.60; 95% CI: 1.00-2.55; p=0.0496), PB-type PCBs (HR = 1.64; 95% CI: 1.01-2.68; p=0.0472), and mixed-type PCBs (HR = 1.53; 95% CI: 0.98-2.39; p=0.0621) (Table V). However, after additionally adjusting for comorbid hypertension (Model 2), CHD hazard ratio estimates for a 2-fold increase in PCBs became slightly larger in magnitude and reached statistical significance for both total PCBs (HR = 1.68; 95% CI: 1.05-2.69; p=0.0306) and PB-type PCBs (HR = 1.72; 95% CI: 1.06-2.81; p=0.0294) (Table V). Further adjusting for diabetes

TABLE II. COHORT CHARACTERISTICS BY CHD-RELATED OUTCOMES DURING FOLLOW UP [a]

Variable [b]	Study Sample	Coronary Heart Disease		Myocardial Infarction		Angina Pectoris	
	n (%)	*Incidence*	*p-value* [c]	*Incidence*	*p-value* [c]	*Incidence*	*p-value* [c]
Overall	819 (100.0)	8.2	-	5.2	-	3.8	-
Sex							
Male	543 (66.3)	12.2	<0.001	7.1	0.0021	5.2	0.0097
Female	276 (33.7)	1.1	Ref.	1.5	Ref.	1.1	Ref.
Age Group							
20-39	174 (21.2)	1.2	Ref.	0.6	Ref.	0.6	Ref.
40-59	562 (68.6)	9	0.1561	5.7	0.1709	4.3	0.2212
60-75	83 (10.1)	19.4	<0.001	11.8	0.0016	7.6	0.0142
BMI Category							
Norm/Under	263 (32.1)	3.2	Ref.	3.9	Ref.	1.6	Ref.
Overweight	380 (46.4)	9.3	0.2451	5.2	0.9224	3.8	0.7604
Obese	176 (21.5)	14.1	0.0009	7.3	0.1514	7.2	0.0061
Smoking							
Non-smokers	507 (62.0)	7.6	Ref.	5.1	Ref.	3.1	Ref.
Smokers	312 (38.0)	9.3	0.4788	5.3	0.9052	4.8	0.2624
Education							
Less than HS	298 (36.4)	7.7	Ref.	6.7	Ref.	4.2	Ref.
HS or Equiv.	270 (32.9)	7.9	0.8016	3.5	0.1544	2.7	0.2585
Some College	251 (30.7)	9.1	0.538	5.3	0.8026	4.5	0.4549
Hypertension							
Never	463 (56.5)	3.6	Ref.	2.7	Ref.	1.3	Ref.
Ever	356 (43.5)	14.1	<0.001	8.6	0.0004	7.1	0.0002
Diabetes							
Never	693 (85.0)	6.9	Ref.	4.2	Ref.	2.7	Ref.
Ever	122 (15.0)	15.5	0.0036	11.5	0.0019	10.5	0.0002
Total Lipids							
1st Tertile	273 (33.3)	3.6	Ref.	3.3	Ref.	2.7	Ref.
2nd Tertile	273 (33.3)	6.7	0.8734	5.4	0.7756	2.9	0.6258
3rd Tertile	273 (33.3)	14.9	0.0444	7	0.2821	5.8	0.1995
Any Fish Meals							
None to <0.5 per week	348 (42.4)	6	Ref.	5.3	Ref.	5.3	Ref.
0.5 to <2 per week	448 (54.7)	9.9	0.5681	5.3	0.9634	2.6	0.2663
≥2 per week	23 (2.9)	10.4	0.6643	0	0.9634	4.4	0.8478
Great Lakes Fish Meals							
None to <0.5 per week	485 (59.2)	7	Ref.	5.5	Ref.	3.8	Ref.
0.5 to <2 per week	291 (35.5)	10.4	0.2167	5	0.5745	3.9	0.6591
≥2 per week	43 (5.3)	5.9	0.5777	2.4	0.4317	2.4	0.6403
Family History of MI							
No	772 (88.2)	8.3	Ref.	4.7	Ref.	3.8	Ref.
Yes	97 (11.8)	7.1	0.6874	8.9	0.1001	3.4	0.827
Family History of CHD							
No	517 (63.1)	6.4	Ref.	3.4	Ref.	2.6	Ref.
Yes	302 (36.9)	11.1	0.0268	8.4	0.0034	5.9	0.0236
Family History of Diabetes							
No	537 (65.6)	8.6	Ref.	5	Ref.	3.1	Ref.
Yes	282 (34.4)	7.5	0.6195	5.6	0.7874	5.2	0.1387

[a] BMI = body mass index; MI = myocardial infarction; CHD = coronary heart disease, HS = high school

[b] All characteristics were ascertained at baseline except for hypertension and diabetes, which are defined as having been diagnosed at any point during follow-up.

[c] p-value for Chi-square of difference in proportion from reference category

TABLE III. TIME TO EVENT STATISTICS OVERALL AND BY STUDY GROUP [a]

Outcome	Group	Total Follow-up Time [b]		Event	Censored
		N	*Median (25th, 75th)*	*n (%)*	*n (%)*
CHD	**Overall**	733	15.6 (10.4, 22.3)	60 (8.2)	520 (70.9)
	Captains	518	15.6 (10.1, 22.4)	47 (9.1)	378 (73.0)
	WI Anglers	83	15.8 (15.4, 22.8)	5 (6.0)	49 (59.0)
	Referents	132	15.3 (9.5, 21.8)	8 (6.1)	93 (70.5)
MI	**Overall**	788	15.5 (10.1, 22.0)	41 (5.2)	570 (72.3)
	Captains	561	15.5 (10.0, 22.2)	31 (5.5)	416 (74.2)
	WI Anglers	85	15.9 (15.4, 22.8)	3 (3.5)	51 (60.0)
	Referents	142	14.9 (9.4, 21.7)	7 (4.9)	103 (72.5)
Angina	**Overall**	792	15.6 (10.1, 22.0)	30 (3.8)	573 (72.4)
	Captains	564	15.6 (10.1, 22.3)	20 (3.6)	416 (73.8)
	WI Anglers	84	15.8 (15.4, 22.8)	3 (3.6)	51 (60.7)
	Referents	144	14.9 (9.4, 21.6)	7 (4.9)	106 (73.6)

[a] CHD = coronary heart disease; MI = myocardial infarction; WI = Wisconsin
[b] Total follow-up time was calculated as time from baseline PCB measurement to either time of event or last CHD follow-up

(Model 3) did not affect the magnitude or precision of model estimates while removing adjustment for serum lipids (Model 4) resulted in null associations (Table V).

There remained no associations between any PCB grouping and MI or angina in any of the 4 adjusted models (Table V). While total fish meals did not confound the associations of PCBs with CHD or related outcomes, the combination of Great Lakes fish and nearest Great Lake to the participant's residence was a negative confounder of the observed associations, increasing the magnitude of estimates by nearly 30% (results not shown).

Serum DDE was not associated with time to diagnosis of CHD, MI, or angina in crude proportional hazard regression models and hazard ratio estimates were further attenuated after adjusting for potential confounders (results not shown). There was no interaction of any variable

TABLE IV. GEOMETRIC MEANS LEVELS OF BASELINE PCB ANALYTES BY COHORT CHARACTERISTICS [a]

Variable [b]	**Total PCBs**		**MC-type PCBs**		**PB-type PCBs**		**Mixed-type PCBs**	
	Geomean (95% CI)	*p-value* [c]	*Geomean (95% CI)*	*p-value* [c]	*Geomean (95% CI)*	*p-value* [c]	*Geomean (95% CI)*	*p-value* [c]
Overall	3.99 (3.80, 4.20)	-	0.39 (0.35, 0.42)	-	2.27 (2.16, 2.39)	-	1.29 (1.22, 1.37)	-
Sex								
Female	3.06 (2.85, 3.27)	Ref.	0.33 (0.3, 0.37)	Ref.	1.71 (1.6, 1.83)	Ref.	0.99 (0.91, 1.07)	Ref.
Male	4.58 (4.28, 4.89)	<0.0001	0.42 (0.37, 0.47)	0.0012	2.62 (2.45, 2.81)	<0.0001	1.48 (1.37, 1.60)	<0.0001
Age Group								
20-39	3.05 (2.76, 3.37)	Ref.	0.3 (0.26, 0.36)	Ref.	1.73 (1.57, 1.9)	Ref.	1.00 (0.88, 1.13)	Ref.
40-59	4.19 (3.96, 4.44)	<0.0001	0.41 (0.37, 0.45)	0.0004	2.38 (2.25, 2.53)	<0.0001	1.36 (1.27, 1.45)	<0.0001
60-75	5.06 (4.43, 5.78)	<0.0001	0.48 (0.39, 0.59)	<0.0001	2.9 (2.55, 3.3)	<0.0001	1.62 (1.40, 1.80)	<0.0001
BMI Category								
Norm/Under	3.43 (3.19, 3.69)	Ref.	0.33 (0.3, 0.37)	Ref.	1.96 (1.82, 2.11)		1.11 (1.01, 1.21)	Ref.
Overweight	4.29 (4.00, 4.60)	<0.0001	0.4 (0.36, 0.45)	0.0032	2.46 (2.29, 2.65)	<0.0001	1.38 (1.28, 1.49)	<0.0001
Obese	4.29 (3.87, 4.76)	0.0004	0.46 (0.39, 0.54)	0.0003	2.38 (2.15, 2.63)	0.0018	1.42 (1.26, 1.59)	0.0003
Smoking								
Non-smokers	3.95 (3.69, 4.22)	Ref.	0.38 (0.34, 0.43)	Ref.	2.32 (2.15, 2.49)	Ref.	1.34 (1.23, 1.46)	Ref.
Smokers	4.07 (3.79, 4.38)	0.5321	0.39 (0.35, 0.44)	0.5527	2.24 (2.1, 2.4)	0.5289	1.27 (1.17, 1.37)	0.3218
Education								
Less than HS	3.95 (3.67, 4.26)	Ref.	0.38 (0.34, 0.42)	Ref.	2.25 (2.09, 2.42)	Ref.	1.28 (1.17, 1.39)	Ref.
HS or Equiv.	4.08 (3.77, 4.42)	0.5416	0.41 (0.36, 0.46)	0.2318	2.29 (2.12, 2.48)	0.7114	1.34 (1.22, 1.47)	0.4391
Some College	3.95 (3.61, 4.32)	0.985	0.38 (0.33, 0.44)	0.904	2.27 (2.07, 2.49)	0.8593	1.27 (1.14, 1.40)	0.8768
Hypertension								
Never	3.76 (3.53, 4.00)	Ref.	0.36 (0.33, 0.4)	Ref.	2.13 (2, 2.27)	Ref.	1.22 (1.13, 1.31)	Ref.
Ever	4.33 (4.03, 4.65)	0.0021	0.42 (0.37, 0.48)	0.0142	2.46 (2.29, 2.65)	0.002	1.39 (1.28, 1.52)	0.0076

[a] MC = 3-methylcholanthrene; PB = phenobarbital; Geomean = geometric mean; CI = confidence interval; BMI = body mass index; HS = high school

[b] All characteristics were ascertained at baseline except for hypertension and diabetes, which are defined as having been diagnosed at any point during follow-up.

[c] p-value for t-test of difference in log-mean from reference category

TABLE IV (CONTINUED). GEOMETRIC MEANS LEVELS OF BASELINE PCB ANALYTES BY COHORT CHARACTERISTICS [a]

Variable [b]	Total PCBs		MC-type PCBs		PB-type PCBs		Mixed-type PCBs	
	Geomean (95% CI)	*p-value* [c]	*Geomean (95% CI)*	*p-value* [c]	*Geomean (95% CI)*	*p-value* [c]	*Geomean (95% CI)*	*p-value* [c]
Diabetes								
Never	3.92 (3.72, 4.13)	Ref.	0.37 (0.34, 0.41)	Ref.	2.23 (2.12, 2.36)	Ref.	1.27 (1.19, 1.35)	Ref.
Ever	4.53 (4.02, 5.11)	0.0247	0.48 (0.04, 0.57)	0.0087	2.53 (2.25, 2.84)	0.0517	1.49 (1.30, 1.70)	0.0203
Total Lipids								
1st Tertile	3.59 (3.04, 4.23)	Ref.	0.36 (0.29, 0.46)	Ref.	2.03 (1.73, 2.38)	Ref.	1.16 (0.96, 1.39)	Ref.
2nd Tertile	3.89 (3.53, 4.30)	0.3733	0.38 (0.33, 0.43)	0.7725	2.21 (2, 2.43)	0.3586	1.26 (1.14, 1.40)	0.3531
3rd Tertile	4.55 (3.96, 5.23)	0.055	0.43 (0.36, 0.51)	0.3357	2.6 (2.25, 3)	0.0345	1.48 (1.28, 1.70)	0.0759
Any Fish Meals								
None to <0.5 per week	3.63 (3.38, 3.89)	Ref.	0.35 (0.31, 0.4)	Ref.	2.07 (1.93, 2.22)	Ref.	1.17 (1.08, 1.27)	Ref.
0.5 to <2 per week	4.25 (3.99, 4.53)	0.0006	0.41 (0.37, 0.46)	0.0207	2.41 (2.26, 2.57)	0.0006	1.38 (1.28, 1.48)	0.0013
≥2 per week	5.07 (3.61, 7.12)	0.0184	0.54 (0.34, 0.85)	0.035	2.82 (2.02, 3.93)	0.0263	1.66 (1.16, 2.38)	0.0233
Great Lakes Fish Meals								
None to <0.5 per week	3.61 (3.4, 3.84)	Ref.	0.35 (0.32, 0.39)	Ref.	2.07 (1.95, 2.19)	Ref.	1.16 (1.08, 1.25)	Ref.
0.5 to <2 per week	4.57 (4.23, 4.94)	<0.0001	0.44 (0.38, 0.49)	0.0022	2.58 (2.38, 2.8)	<0.0001	1.49 (1.37, 1.63)	<0.0001
≥2 per week	4.99 (4.05, 6.15)	0.0009	0.49 (0.38, 0.65)	0.0131	2.76 (2.23, 3.4)	0.0025	1.65 (1.32, 2.07)	0.0012
Family History of MI								
No	4.02 (3.82, 4.24)	Ref.	0.38 (0.34, 0.42)	Ref.	2.29 (2.15, 2.44)	Ref.	1.30 (1.22, 1.39)	Ref.
Yes	3.78 (3.34, 4.28)	0.3597	0.4 (0.35, 0.46)	0.4272	2.24 (2.08, 2.41)	0.6196	1.22 (1.06, 1.4)	0.3488
Family History of CHD								
No	4.02 (3.78, 4.27)	Ref.	0.39 (0.36, 0.43)	Ref.	2.29 (2.17, 2.41)	Ref.	1.30 (1.22, 1.40)	Ref.
Yes	3.95 (3.67, 4.26)	0.7122	0.36 (0.3, 0.44)	0.4479	2.16 (1.91, 2.45)	0.412	1.28 (1.17, 1.39)	0.6907
Family History of Diabetes								
No	4.00 (3.78, 4.24)	Ref.	0.38 (0.35, 0.42)	Ref.	2.28 (2.15, 2.41)	Ref.	1.29 (1.21, 1.39)	Ref.
Yes	3.99 (3.67, 4.33)	0.9428	0.4 (0.35, 0.45)	0.5534	2.26 (2.08, 2.45)	0.8494	1.29 (1.18, 1.41)	0.984

[a] MC = 3-methylcholanthrene; PB = phenobarbital; Geomean = geometric mean; CI = confidence interval; BMI = body mass index; HS = high school

[b] All characteristics were ascertained at baseline except for hypertension and diabetes, which are defined as having been diagnosed at any point during follow-up.

[c] p-value for t-test of difference in log-mean from reference category

TABLE V. MODEL-ADJUSTED RISK OF INCIDENT DIAGNOSIS OF CHD-RELATED HEALTH OUTCOMES WITH DOUBLING OF SERUM PCBS [a]

PCB Grouping	Endpoint	Model 0 [b] Crude Model		Model 1 [b] Base Model		Model 2 [b] Base + Hypertension		Model 3 [b] Base + Diabetes		Model 4 [b] Base Without Lipids	
		HR (95% CI) [c]	p-value	HR (95% CI) [c]	p-value	HR (95% CI) [c]	p-value	HR (95% CI) [c]	p-value	HR (95% CI) [c]	p-value
Total PCBs	*CHD*	1.74 (1.17-2.56)	0.0058	1.60 (1.00-2.55)	0.0496	1.68 (1.05-2.69)	0.0306	1.59 (1.00-2.54)	0.0518	1.36 (0.85-2.19)	0.2020
	MI	1.36 (0.85-2.17)	0.1991	1.25 (0.72-2.17)	0.4169	1.29 (0.73-2.25)	0.3788	1.25 (0.72-2.17)	0.4188	1.10 (0.64-1.87)	0.7379
	Angina	1.51 (0.88-2.59)	0.1306	1.27 (0.70-2.31)	0.4328	1.26 (0.69-2.29)	0.4545	1.28 (0.70-2.33)	0.4266	1.22 (0.65-2.27)	0.5307
MC-type PCBs	*CHD*	1.26 (0.93-1.71)	0.1350	1.15 (0.82-1.62)	0.4126	1.16 (0.81-1.66)	0.4244	1.15 (0.82-1.63)	0.4124	1.10 (0.79-1.53)	0.5794
	MI	1.01 (0.70-1.47)	0.9390	0.96 (0.63-1.46)	0.8412	0.93 (0.61-1.43)	0.7463	0.96 (0.63-1.46)	0.8398	0.91 (0.61-1.36)	0.6577
	Angina	1.15 (0.73-1.79)	0.5490	0.99 (0.63-1.56)	0.9608	0.95 (0.59-1.52)	0.8283	0.99 (0.63-1.55)	0.9507	0.99 (0.63-1.56)	0.9711
PB-type PCBs	*CHD*	1.78 (1.20-2.65)	0.0045	1.64 (1.01-2.68)	0.0472	1.72 (1.06-2.81)	0.0294	1.63 (1.00-2.66)	0.0501	1.38 (0.84-2.27)	0.2008
	MI	1.41 (0.88-2.27)	0.1508	1.30 (0.74-2.28)	0.3598	1.35 (0.76-2.38)	0.3071	1.30 (0.74-2.28)	0.3616	1.12 (0.65-1.95)	0.6808
	Angina	1.53 (0.90-2.61)	0.1144	1.30 (0.71-2.39)	0.3900	1.30 (0.71-2.38)	0.4012	1.32 (0.71-2.42)	0.3787	1.24 (0.66-2.33)	0.4956
Mixed-type PCBs	*CHD*	1.64 (1.12-2.39)	0.0107	1.53 (0.98-2.39)	0.0621	1.60 (1.02-2.52)	0.0427	1.53 (0.98-2.40)	0.0625	1.34 (0.86-2.09)	0.1943
	MI	1.36 (0.85-2.18)	0.1987	1.31 (0.77-2.22)	0.3153	1.34 (0.78-2.28)	0.2889	1.31 (0.77-2.22)	0.3167	1.16 (0.69-1.95)	0.5691
	Angina	1.46 (0.86-2.46)	0.1590	1.22 (0.69-2.16)	0.4943	1.21 (0.68-2.14)	0.5212	1.22 (0.69-2.17)	0.4948	1.19 (0.65-2.15)	0.5691

[a] PCB = polychlorinated biphenyl, CYP = cytochrome P-450, CHD = coronary heart disease, MI = myocardial infarction, HR = hazard ratio, CI = confidence interval

[b] PCBs were log-transformed in all models. The base model (Model 1) was adjusted for sex, age (quadratic), time-varying BMI (quadratic), time-varying Great Lakes fish meals (quadratic), nearest Great Lake, time-varying Great Lakes fish meals*nearest Great Lake (interaction), time-varying log-transformed total lipids, family history of CHD, family history of MI, and family history of diabetes. Models 2-4 include modifications to the base model.

[c] Hazard ratios are interpreted as the ratio of risk for a 2-fold increase in PCBs

with DDE or PCBs in associations with CHD-related outcomes (results not shown). Male only model estimates were slightly attenuated with reduced precision while there were too few diagnoses for female only models (results not shown)

3.4. Discussion

This study demonstrates that long-term dietary exposure to certain types of PCBs may be associated with increased risk of CHD in a high-risk population of Great Lakes sport-caught fish consumers, while exposure to DDE did not appear to be associated with risk of heart disease.

Our findings of a significant association of PB-type PCBs with elevated risk of CHD are consistent with the literature, which generally suggests an association of PCBs with CHD-related outcomes. However, the literature is highly variable with respect to exposure ascertainment methods, PCB congener classification methods, CHD-related outcome of interest, and study design.

Despite methodologic differences in the literature, the direction and magnitude of our estimate for the association of PCBs with CHD is consistent with three previous studies. A cross-sectional study of 335 adult native North American Akwesanse Mohawks, using a comprehensive series of causal models accounting for the effects of BMI, age, serum lipids, and PCBs, found a statistically significant ($p=0.01$) positive association of with PCBs with self-reported cardiovascular disease (34). A prospective study of 1,016 Swedish elderly males and females reported statistically significant ($p<0.05$) associations of PCBs with three markers of atherosclerotic progression with particularly strong associations using total PCBs, TEQs, and highly chlorinated PCBs (25). An ecologic study of hospital discharge in highly contaminated residences along the Hudson River in New York reported 36% higher odds (95% CI: 1.19-1.56)

of hospital discharge for CHD in areas highly polluted with PCBs as compared to all other areas of New York (except New York City) (24).

In contrast with our study, a cross-sectional study of the 889 males and females age 20 and older in the general US population (NHANES 1999-2002) reported 5.0 (95% CI: 1.2-20.4; $p<0.01$) times greater odds of self-reported cardiovascular disease in the highest quartile of dioxin-like PCBs as compared to the lowest quartile and 3.8 (95% CI: 1.1-12.8; $p=0.01$) times greater odds of self-reported cardiovascular disease in the highest quartile of non-dioxin-like PCBs as compared to the lowest quartile. However, these associations were observed only among females with much smaller and non-significant associations in males (49), while we were unable to estimate a difference in associations between males and females due to the small number of female participants in the study.

Our hazard ratio estimates for the association of PCBs with incident MI were imprecise and non-significant at traditional alpha of 0.05, but the direction and magnitude of the estimate is consistent with what limited literature is available. Two population-based studies of Swedish middle-aged and elderly males and females, including over 30,000 males and over 30,000 females, reported significantly increased risk of myocardial infarction with increasing dietary estimates of PCBs exposure. Specifically, among females, the study reported a hazard ratio of 1.74 (1.30-2.33) for risk of myocardial infarction in the highest quartile of PCBs as compared to the lowest quartile. Similarly, among males, the second study reported a hazard ratio of 1.58 (95% CI 1.10-2.25) for risk of myocardial infarction in the highest quintile of PCBs compared to the lowest quintile. However, in the second study, after stratifying by waist circumference, the association was observed only in non-obese males. In the same previously mentioned ecologic study of hospital discharge in highly contaminated residences along the Hudson River in New

York, results showed 39% higher odds (95% CI: 1.19-1.63) of hospital discharge for acute myocardial infarction in areas highly polluted with PCBs as compared to all other areas of New York (except New York City) (24).

While we did not have data on omega-3 fatty acid intake, we were able to use total fish meals as a proxy. However, total fish meals were not associated with any CHD-related outcome, did not confound any of the main associations, and were thus removed from the final models. Great Lakes fish meals was included in the final model due to slight increase of the main associations, suggesting that the beneficial nutritional effects of fish consumption on CHD-related outcomes was partially captured by this variable. However, use of total or Great Lakes fish meals as a proxy for omega-3 fatty acid intake may have resulted in misclassification of omega-3 fatty acid intake since type of fish consumed and other sources of omega-3 fatty acids were not considered. Furthermore, the 2016 Bergkvist et al. study restricted to males and reported a significant association only among males with waist circumference less than 102cm. Although we did not have information on waist circumference, we did not observe any effect modification by BMI.

To our knowledge, this is the first study investigating the association of PCBs with incident diagnosis of angina pectoris as an independent outcome, so there is no comparability to the literature. However, the magnitude of the hazard ratio estimate for angina pectoris is comparable to our estimate for CHD as well as other estimates of CHD or CVD in the literature. Due to lack of statistical power, our hazard ratio estimates for this association was imprecise and failed to reach statistical significance.

There is substantial literature demonstrating an association of elevated PCBs with increased risk of both hypertension and diabetes, although the associations tend to be weak and

the methods inconsistent for both. Furthermore, both hypertension and diabetes are known risk factors for CHD making them both plausible mediators in the associations of PCBs with CHD-related outcomes. However, to our knowledge, this is the first study directly addressing the roles of hypertension and diabetes in these associations. After adjusting for hypertension in Model 2, estimates of the main associations increased, which suggests slight negative confounding, not mediation, by hypertension. In contrast, adjusting for diabetes in Model 3 did not change the estimates of the base model, indicating that the association of PCBs with CHD is not mediated by diabetes. Thus, there is no evidence to support mediation or substantial confounding by either hypertension or diabetes.

The literature has consistently demonstrated that serum lipids play an important role in the measurement of POPs. Furthermore, lipid metabolism and PCB metabolism may be modified by metabolic conditions such as diabetes. Since hyperlipidemia is a known risk factor for CHD and CHD-related outcomes, lipids may potentially act as a mediator or a confounder. Our results suggest strong negative confounding of these associations by total serum lipids, as removal of total lipid adjustment in Model 3 resulted in attenuated non-significant associations.

Total Great Lakes fish meals were not associated with any CHD-related outcome, even after adjusted for known confounders in longitudinal models. The literature is consistent in demonstrating a protective effect of fish consumption on CHD incidence and mortality. However, in part due to high missingness and extensive imputations of Great Lakes fish meals, adjusted estimates of the relationship between Great Lakes fish consumption and CHD-related outcomes did not reach statistical significance and further adjustment for PCBs did not modify these associations. However, Great Lakes fish meals did confound the main associations of PCBs

with CHD-related outcomes, and confounding was substantially stronger after accounting for the specific Great Lake from which fish were likely to have been harvested.

Risk of CHD was most strongly associated with PB-type PCB congeners. Previous studies have had much difficulty addressing simultaneous exposure to multiple PCB congeners over a long period of time. Since PCB congeners are highly correlated, statistical models cannot estimate associations for all congeners in a single model. Much of the previous toxicological literature aimed at grouping of PCBs based on mechanism of action has focused on the estrogenic and antiestrogenic properties of PCBs (50,51), but more recent toxicological literature has suggested that the proposed action of PCBs on hormones is a downstream effect through induction of metabolic CYP enzymes, which play a role in both PCB metabolism and sex hormone metabolisms.

In observational studies, testosterone appears to play a role in protection against CHD in males while elevated estrogen may be harmful (52,53) Furthermore, while there is evidence of increased risk of CHD in males receiving androgen deprivation therapy for treatment of prostate cancer, but results from clinical trials are inconclusive (54)(55). Across all ages and multiple populations, researchers have observed an increased risk of CHD mortality in males compared to females (56), which has been largely attributed to differences in circulating sex hormones. In contrast to males, elevated estrogens may reduce risk of CHD in females (57,58).

The mechanism through which sex hormones modify cardiovascular risk is not clear, but it likely occurs through modification of risk factors such as lipid profile and induction of a chronic state of systemic inflammation. Inflammation plays a major role in atherosclerotic progression. Many chronic diseases have been known to induce a chronic state of systemic inflammation which is suggested to more rapidly advance the atherosclerotic progression and increase risk of CHD

(59)(60). Given the evidence of relationships between PCBs, hormones, and CHD, it is plausible that PB-type PCB congeners may be increasing risk of CHD through elevation of circulating endogenous hormones.

As with any observational study, this study has its limitations. All data on health-related states were self-reported history of physician diagnosis. Due to imperfect recall by participants, we expected some degree of misclassification of CHD-related outcomes with respect to both diagnosis status and time of diagnosis. However, misclassification of CHD-related diagnoses is not expected to be related to PCB exposure. Non-differential misclassification would likely result in an attenuation of the true estimate and thus the true estimate may be higher than what we have reported in this study. Fish consumption was also self-reported and is subject to the same limitations.

Limited resources prevented us from measuring PCBs in the entire cohort, which substantially restricted the sample size of our study. Furthermore, chronic diseases like CHD and CHD-related outcomes typically do not develop until later in life. Since the average age of the cohort at baseline was young, a long period of time was required to begin observing diagnoses of CHD-related outcomes. Although we allowed up to 24 years of follow-up, most participants were lost to follow-up (censored) before reaching an age at which CHD-related outcomes are likely to be diagnosed. This resulted in a low number of diagnoses which, along with the small sample size, limited the power to detect differences in risk between the highly exposed and the less exposed. For example, in 2010, the average age at first at first MI for males and females in the United States was 64.5 and 70.3, respectively (1). In this study, the average age at baseline was 47 years and most participants were followed for 15.5 years. Thus, only 198 (36.5%) males and 14 (5.2%) females have reached the national average age at first MI before their last follow-

up. The lack of expected cases can be seen in the considerably lower observed incidence of CHD in our study (6.2 per 1000 person-years) compared to the national average (9.6 per 1000 person-years) (1).

Limited resources also prevented us from consistently collecting data on current medication use at all time points. Lack of information on important drugs used to treat CHD and other comorbid conditions (i.e. lipid-lowering drugs, antihypertensive drugs, beta blockers, and calcium channel blockers) limited our ability to additionally identify or confirm diagnosis of CHD and related outcomes as well as other comorbid conditions that may play a role in these associations.

While Great Lakes fish consumption is a major contributor to both DDE and PCB exposure, it is also a source of methyl-mercury (61). Exposure to methyl-mercury has also been associated with increased risk of CHD-related outcomes and is thus a potential confounder in the association of POPs with CHD (62). Since we did not ascertain mercury exposure in this study, residual confounding by methyl-mercury exposure may result in biased estimates of the main associations. Exposure to other POPs such as dioxins, furans, and dioxin-like PCBs are also possible sources of residual confounding, subject to the same potential biases.

Since serum POPs were not measured in the same participants at all follow-ups, there is a large proportion of missing data. To address this, we imputed all missing data using fully conditional specification of multiple imputations by chained equations. While this method allowed us to recover observed data from participants that would otherwise have been deleted from the analysis due to missing data, it also introduced additional variability and imprecision in variables with high missingness and poor prediction. High missingness was also observed in self-reported fish meals, which are subject to the same issues with high variability due to imputations.

As with all longitudinal cohorts, many participants were lost to follow-up either due to voluntary non-participation or death. Charter boat captains had a vested interest in the study and were more likely to respond to follow-up surveys and offer follow-up blood samples than referents. As a result, we observed more frequent drop out in referents compared to charter boat captains (11). Charter boat captains also eat Great Lakes sport-caught fish more frequently than referents resulting in elevated serum POPs relative to referents (63). Additionally, participants diagnosed with CHD or related outcomes may also be less likely to participate due to illness or death prior to follow-up. Since both exposure and outcome may influence selection into the study, selection bias may be introduced. However, the direction and magnitude of selection bias on the main associations is unclear.

Despite its limitations, this study benefits from a longitudinal design spanning 24 years of follow-up as well as multiple measurements of DDE and PCB exposure biomarkers and Great Lakes fish consumption over the course of the study. Results from our longitudinal study support a positive association of PCBs with risk of CHD-related outcomes, which is supported by previously conducted cross-sectional studies in the literature. However, since we have established that PCB exposure preceded all CHD-related outcomes in this study, our results additionally provide additional insight into the temporality of PCD exposure and CHD-related outcomes. While much of the exposure data was missing, the computationally complex imputation methods allowed us to recover missing information and include participants with missing data that would otherwise have been excluded from the analysis, which greatly increased the statistical power of the study. Finally, with multiple comprehensive health and fish consumption questionnaires, we were able to adjust for many known confounders of these associations resulting in a maximally precise and minimally biased estimate of these

associations. While these methods greatly increased statistical power, additional longitudinal studies will be necessary to confirm these results. Future research should focus on plausible mechanisms of action in these associations, such as increased oxidative stress and sex hormone disruption.

3.5. Conclusions

This prospective study demonstrated that exposure to PCBs may be associated with increased cardiovascular risk in a population of frequent and infrequent Great Lakes sport-caught fish consumers. Specifically, PCB congeners which are phenobarbital-type inducers of CYP2B1, CYP2B2, and CYP2A showed the strongest increase in risk of CHD, suggesting a mechanism of action related to metabolic processes involving these CYP groups. Longitudinal studies are needed to confirm these results and future studies should also investigate potential mechanisms of action, include more diverse populations, and assess additional chemicals exposures related to fish consumption, such as metals and polyfluoroalkyl substances, as well as beneficial nutrients.

Cited Literature

1. Lloyd-Jones D, Adams RJ, Brown TM, et al. Heart disease and stroke statistics--2010 update: A report from the American Heart Association. *Circulation*. 2010;121(7):e46-e215.

2. American Heart Association. Fish and omega-3 fatty acids. http://www.heart.org/HEARTORG/GettingHealthy/NutritionCenter/HealthyEating/Fish-101_UCM_305986_Article.jsp#aha_recommendation. Updated 2014. Accessed 4/15, 2014.

3. Daviglus ML, Stamler J, Orencia AJ, et al. Fish consumption and the 30-year risk of fatal myocardial infarction. *N Engl J Med*. 1997;336(15):1046-1053.

4. Zheng J, Huang T, Yu Y, Hu X, Yang B, Li D. Fish consumption and CHD mortality: An updated meta-analysis of seventeen cohort studies. *Public Health Nutr*. 2012;15(04):725-737.

5. Oomen CM, Feskens EJ, Rasanen L, et al. Fish consumption and coronary heart disease mortality in Finland, Italy, and the Netherlands. *Am J Epidemiol*. 2000;151(10):999-1006.

6. Iso H, Kobayashi M, Ishihara J, et al. Intake of fish and n3 fatty acids and risk of coronary heart disease among Japanese: The Japan public health center-based (JPHC) study cohort I. *Circulation*. 2006;113(2):195-202.

7. Kwak SM, Myung S, Lee YJ, Seo HG, Korean Meta-analysis Study Group. Efficacy of omega-3 fatty acid supplements (eicosapentaenoic acid and docosahexaenoic acid) in the secondary prevention of cardiovascular Disease: A meta-analysis of randomized, double-blind, placebo-controlled. *Arch Intern Med*. 2012;172(9):686-694.

8. Turyk ME, Bhavsar SP, Bowerman W, et al. Risks and benefits of consumption of Great Lakes fish. *Environ Health Perspect*. 2012;120(1):11.

9. Imm P, Knobeloch L, Anderson HA, Great Lakes Sport Fish Consortium. Fish consumption and advisory awareness in the Great Lakes basin. *Environ Health Perspect*. 2005:1325-1329.

10. Knobeloch L, Turyk M, Imm P, Anderson H. Polychlorinated biphenyls in vacuum dust and blood of residents in 20 Wisconsin households. *Chemosphere*. 2012;86(7):735-740.

11. Turyk M, Anderson H, Knobeloch L, Imm P, Persky V. Organochlorine exposure and incidence of diabetes in a cohort of Great Lakes sport fish consumers. *Environ Health Perspect*. 2009;117(7):1076-1082.

12. Lee DH, Lee IK, Song K, et al. A strong dose-response relation between serum concentrations of persistent organic pollutants and diabetes: Results from the national health and examination survey 1999-2002. *Diabetes Care*. 2006;29(7):1638-1644.

13. Everett CJ, Thompson OM. Associations of dioxins, furans and dioxin-like PCBs with diabetes and pre-diabetes: Is the toxic equivalency approach useful? *Environ Res*. 2012;118:107-111.

14. Wu H, Bertrand KA, Choi AL, et al. Persistent organic pollutants and type 2 diabetes: A prospective analysis in the nurses' health study and meta-analysis. *Environ Health Perspect*. 2012;121(2):153-161.

15. Silverstone AE, Rosenbaum PF, Weinstock RS, et al. Polychlorinated biphenyl (PCB) exposure and diabetes: Results from the Anniston community health survey. *Environ Health Perspect*. 2012;120(5).

16. Vena J, Boffetta P, Becher H, et al. Exposure to dioxin and nonneoplastic mortality in the expanded IARC international cohort study of phenoxy herbicide and chlorophenol production workers and sprayers. *Environ Health Perspect*. 1998;106 Suppl 2:645-653.

17. Gustavsson P, Hogstedt C. A cohort study of Swedish capacitor manufacturing workers exposed to polychlorinated biphenyls (PCBs). *Am J Ind Med*. 1997;32(3):234-239.

18. Prince MM, Hein MJ, Ruder AM, Waters MA, Laber PA, Whelan EA. Update: Cohort mortality study of workers highly exposed to polychlorinated biphenyls (PCBs) during the manufacture of electrical capacitors, 1940-1998. *Environ Health*. 2006;5:13.

19. Bertazzi PA, Bernucci I, Brambilla G, Consonni D, Pesatori AC. The Seveso studies on early and long-term effects of dioxin exposure: A review. *Environ Health Perspect*. 1998;106 Suppl 2:625-633.

20. Bertazzi PA, Consonni D, Bachetti S, et al. Health effects of dioxin exposure: A 20-year mortality study. *Am J Epidemiol*. 2001;153(11):1031-1044.

21. Goncharov A, Pavuk M, Foushee HR, Carpenter DO. Blood pressure in relation to concentrations of PCB congeners and chlorinated pesticides. *Environ Health Perspect*. 2011;119(3):319.

22. Li M, Tsai P, Chen P, Hsieh C, Guo YL, Rogan WJ. Mortality after exposure to polychlorinated biphenyls and dibenzofurans: 30 years after the “Yucheng accident”. *Environ Res*. 2013;120:71-75.

23. Tomasallo C, Anderson H, Haughwout M, Imm P, Knobeloch L. Mortality among frequent consumers of Great Lakes sport fish. *Environ Res*. 2010;110(1):62-69.

24. Sergeev AV, Carpenter DO. Hospitalization rates for coronary heart disease in relation to residence near areas contaminated with persistent organic pollutants and other pollutants. *Environ Health Perspect*. 2005;113(6):756-761.

25. Lind PM, van Bavel B, Salihovic S, Lind L. Circulating levels of persistent organic pollutants (POPs) and carotid atherosclerosis in the elderly. *Environ Health Perspect*. 2012;120(1):38-43.

26. Bergkvist C, Berglund M, Glynn A, Wolk A, Åkesson A. Dietary exposure to polychlorinated biphenyls and risk of myocardial infarction–A population-based prospective cohort study. *Int J Cardiol*. 2015.

27. Valera B, Jørgensen ME, Jeppesen C, Bjerregaard P. Exposure to persistent organic pollutants and risk of hypertension among Inuit from Greenland. *Environ Res*. 2013;122:65-73.

28. Valera B, Ayotte P, Poirier P, Dewailly É. Associations between plasma persistent organic pollutant levels and blood pressure in Inuit adults from Nunavik. *Environ Int*. 2013;59:282-289.

29. Everett CJ, Mainous III AG, Frithsen IL, Player MS, Matheson EM. Association of polychlorinated biphenyls with hypertension in the 1999–2002 national health and nutrition examination survey. *Environ Res*. 2008;108(1):94-97.

30. Ha MH, Lee D, Son H, Park S, Jacobs D. Association between serum concentrations of persistent organic pollutants and prevalence of newly diagnosed hypertension: Results from the national health and nutrition examination survey 1999–2002. *J Hum Hypertens*. 2008;23(4):274-286.

31. Huang X, Lessner L, Carpenter DO. Exposure to persistent organic pollutants and hypertensive disease. *Environ Res*. 2006;102(1):101-106.

32. Singh K, Chan HM. Persistent organic pollutants and diabetes among Inuit in the Canadian arctic. *Environ Int*. 2017;101:183-189.

33. Zong G, Valvi D, Coull B, et al. Persistent organic pollutants and risk of type 2 diabetes: A prospective investigation among middle-aged women in nurses' health study II. *Environ Int*. 2018;114:334-342.

34. Goncharov A, Haase RF, Santiago-Rivera A, et al. High serum PCBs are associated with elevation of serum lipids and cardiovascular disease in a native American population. *Environ Res*. 2008;106(2):226-239.

35. Penell J, Lind L, Salihovic S, van Bavel B, Lind PM. Persistent organic pollutants are related to the change in circulating lipid levels during a 5 year follow-up. *Environ Res*. 2014;134:190-197.

36. Singh K, Chan HM. Association of blood polychlorinated biphenyls and cholesterol levels among Canadian Inuit. *Environ Res*. 2018;160:298-305.

37. Hansen LG. Stepping backward to improve assessment of PCB congener toxicities. *Environ Health Perspect*. 1998;106 Suppl 1:171-189.

38. Jansen HT, Cooke PS, Porcelli J, Liu T, Hansen LG. Estrogenic and antiestrogenic actions of PCBs in the female rat: In vitro and in vivo studies. *Reproductive Toxicology*. 1993;7(3):237-248.

39. Hany J, Lilienthal H, Sarasin A, et al. Developmental exposure of rats to a reconstituted PCB mixture or Aroclor 1254: Effects on organ weights, aromatase activity, sex hormone levels, and sweet preference behavior. *Toxicol Appl Pharmacol*. 1999;158(3):231-243.

40. Negri-Cesi P, Colciago A, Pravettoni A, Casati L, Conti L, Celotti F. Sexual differentiation of the rodent hypothalamus: Hormonal and environmental influences. *J Steroid Biochem Mol Biol*. 2008;109(3-5):294-299.

41. Anderson H, Falk C, Fiore B, et al. Consortium for the health assessment of Great Lakes sport fish consumption. *Toxicol Ind Health*. 1996;12(3-4):369-373.

42. Hanrahan LP, Falk C, Anderson HA, Draheim L, Kanarek MS, Olson J. Serum PCB and DDE levels of frequent Great Lakes sport fish consumers—a first look. *Environ Res*. 1999;80(2):S26-S37.

43. Jones RM, Stayner LT, Demirtas H. Multiple imputation for assessment of exposures to drinking water contaminants: Evaluation with the atrazine monitoring program. *Environ Res*. 2014;134:466-473.

44. R Developer Core Team. R: A language and environment for statistical computing. . 2008.

45. Warner J, Osuch JR, Karmaus W, et al. Common classification schemes for PCB congeners and the gene expression of CYP17, CYP19, ESR1 and ESR2. *Sci Total Environ*. 2012;414:81-89.

46. Bernert JT, Turner WE, Patterson DG, Needham LL. Calculation of serum "total lipid" concentrations for the adjustment of persistent organohalogen toxicant measurements in human samples. *Chemosphere*. 2007;68(5):824-831.

47. O'Brien KM, Upson K, Cook NR, Weinberg CR. Environmental chemicals in urine and blood: Improving methods for creatinine and lipid adjustment. . 2015.

48. Maldonado G, Greenland S. Simulation study of confounder-selection strategies. *Am J Epidemiol*. 1993;138(11):923-936.

49. Ha MH, Lee DH, Jacobs DR. Association between serum concentrations of persistent organic pollutants and self-reported cardiovascular disease prevalence: Results from the national health and nutrition examination survey, 1999-2002. *Environ Health Perspect*. 2007;115(8):1204-1209.

50. Wolff MS, Camann D, Gammon M, Stellman SD. Proposed PCB congener groupings for epidemiological studies. *Environ Health Perspect*. 1997;105(1):13-14.

51. Cooke PS, Sato T, Buchanan DL, Robertson L, Hansen L. Disruption of steroid hormone signaling by PCBs. *PCBs Recent Advances in Environmental Toxicology and Health Effects*. 2001:257-263.

52. Corona G, Rastrelli G, Monami M, et al. Hypogonadism as a risk factor for cardiovascular mortality in men: A meta-analytic study. *Eur J Endocrinol*. 2011;165(5):687-701.

53. Ruige JB, Mahmoud AM, De Bacquer D, Kaufman JM. Endogenous testosterone and cardiovascular disease in healthy men: A meta-analysis. *Heart*. 2011;97(11):870-875.

54. Levine GN, D'amico AV, Berger P, et al. Androgen-deprivation therapy in prostate cancer and cardiovascular risk: A science advisory from the American Heart Association, American Cancer Society, and American Urological Association: Endorsed by the American Society for Radiation Oncology. *CA: a cancer journal for clinicians*. 2010;60(3):194-201.

55. Nguyen PL, Je Y, Schutz FA, et al. Association of androgen deprivation therapy with cardiovascular death in patients with prostate cancer: A meta-analysis of randomized trials. *JAMA*. 2011;306(21):2359-2366.

56. Lawlor DA, Ebrahim S, Smith GD. Trends in sex differences in mortality from heart disease: Sex is not same as gender, and theory was first proposed in 1950s, say authors. *BMJ*. 2002;324(7331):237-238.

57. Manson JE, Allison MA, Rossouw JE, et al. Estrogen therapy and coronary-artery calcification. *N Engl J Med*. 2007;356(25):2591-2602.

58. Rossouw JE, Prentice RL, Manson JE, et al. Postmenopausal hormone therapy and risk of cardiovascular disease by age and years since menopause. *JAMA*. 2007;297(13):1465-1477.

59. Emerging Risk Factors Collaboration. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: An individual participant meta-analysis. *The Lancet*. 2010;375(9709):132-140.

60. Pai JK, Pischon T, Ma J, et al. Inflammatory markers and the risk of coronary heart disease in men and women. *N Engl J Med*. 2004;351(25):2599-2610.

61. Bhavsar SP, Gewurtz SB, McGoldrick DJ, Keir MJ, Backus SM. Changes in mercury levels in Great Lakes fish between 1970s and 2007. *Environ Sci Technol*. 2010;44(9):3273-3279.

62. Guallar E, Sanz-Gallardo MI, Veer Pv, et al. Mercury, fish oils, and the risk of myocardial infarction. *N Engl J Med*. 2002;347(22):1747-1754.

63. Hanrahan LP, Falk C, Anderson HA, et al. Serum PCB and DDE levels of frequent Great Lakes sport fish consumers—a first look. *Environ Res*. 1999;80(2):S26-S37.

4. ASSOCIATIONS OVER TIME OF PCBS AND DDE WITH CIRCULATING STEROID SEX HORMONES

4.1. Introduction

Organochlorines are a group of environmental pollutants characterized by a hydrocarbon skeleton and varying degrees of chlorination. These compounds are eliminated slowly compared to other xenobiotics resulting in biologic half-lives of up to a decade (1). This allows pollutants to accumulate and magnify as they move up the food chain, ultimately ending up in humans through consumption of contaminated fish, as well as contaminated meat or dairy products.

Polychlorinated biphenyls (PCBs) are a family of 209 compounds, or congeners, that differ based on the number and position of chlorine atoms replacing hydrogen atoms on their two phenyl rings. PCBs were first produced in the United States in the 1920's with peak production and use in the 1970's until production was banned in 1979 following conclusive evidence of negative health effects as a result of exposure to PCBs. Although PCBs are no longer manufactured in the US, PCBs continue to persist in the environment and in human tissues due to their resistance to degradation and long biological half-lives (Hopf 2013) and ongoing exposure from certain foods such as meat, dairy products, and fatty fish sourced from contaminated waters (Knutsen 2011; Malisch 2014). PCBs produced prior to their ban still exist in the environment as a result of their environmental persistence and many congeners are still measurable in the general US population.

Dichlorodiphenyltrichloroethane is an organochlorine pesticide with widespread use for insect control until its use was banned in the United States in 1973. Due to its environmental persistence, DDT and its metabolites DDE and DDD still exist in the environment, the food chain, and in measurable quantities in the serum of the general US population.

There is a wealth of toxicological and epidemiological literature demonstrating the endocrine-disrupting properties of organochlorines. However, the link to causality is weak due to inconsistencies in methods for PCB exposure ascertainment, selection of key hormones, and analytic methods used to estimate associations. Previous research has primarily focused on relationships between organochlorines and the hypothalamic-pituitary axis, with a particularly focus on their effects on thyroid hormones (2-4). Though less commonly studied, there has also been an interest in relationships between organochlorines and steroid sex hormone disruption.

Steroid sex hormones are generally classified into two groups, androgens and estrogens, though a third class, progestogens, also plays an important role. Under normal conditions, sex steroid hormones remain in physiological balance in both males and females, and disruption of that balance can have short-term effects, such as acne, hirsutism, hair loss, hot flashes, menstrual disorder (females) and erectile dysfunction (males), as well as long-term effects, such as accelerated atherosclerosis, increased risk of cardiovascular disease, and increased risk of early death from stroke, and myocardial infarction.

The biological mechanisms through which organochlorines may modify steroid hormone profiles differs by compound class and even within PCB congeners, as the mechanism depends on the structure of the compound or congener and the group of enzymes which metabolize them. AhR activation by dioxins and dioxin-like PCBs has been well studied (5) and activation of the AhR has been suggested to have anti-androgenic effects (6,7) in males. Coplanar PCBs have strong dioxin-like characteristics which allow them to bind to and activate AhR, though some mono-ortho PCBs can also activate the AhR with lesser potency. Activation of the AhR upregulates synthesis of cytochrome p450s, which then break down PCBs into metabolites containing highly reactive oxygen species. Specifically, coplanar non-ortho substituted (dioxin-

like) PCBs may also illicit an MC-type induction of CYP1A1, CYP1A2, and CYP1B1. While AhR activation is thought to be the primary pathway for coplanar PCBs, both coplanar and non-coplanar PCBs have been shown to have endocrine disrupting properties as well.

Highly chlorinated non-coplanar ortho-substituted (non-dioxin-like) PCBs can elicit a PB-type induction of CYP2B1, CYP2B2, CYP2A1, and CYP3A (8) while lower chlorinated mono-ortho-substituted PCBs are mixed-type inducers, which illicit both MC-type and PB-type responses (8). These enzymes play a critical role in metabolism of xenobiotics such as PCBs. However, they are also involved in synthesis and metabolism of steroid sex hormones. Thus, induction of these enzymes has the potential to modify endogenous sex hormone profiles. Animal models have shown both estrogenic and anti-estrogenic PCB effects through abnormal development of sex organs (9,10) as well as sexual differentiation and behavior in the brain (11).

There is a growing body of literature around in-utero and early childhood exposure to PCBs and their relation to fetal development (12,13), early childhood development (14,15), and sexual maturation (16,17). However, epidemiologic evidence of the relationships between PCBs and circulating sex hormones in adults is sparse and inconsistent. For example, there are 4 previous studies that found an inverse association between PCBs and total testosterone (18-20). In contrast, another 3 studies that found no association between PCBs and total testosterone (2,21,22) and a fourth that found a positive association with a few congeners (23). However, these investigations measured different PCB congeners, employed different strategies for grouping congeners, controlled for different confounders, and infrequently examined modification by biological and demographic factors. Furthermore, the age distribution of participants varied across studies, which could also increase inconsistencies since testosterone and testosterone binding are dramatically influenced by aging (24).

There is substantial evidence from toxicological studies that PCBs and dioxin-like chemicals influence endogenous hormones, and that sex hormones can affect risk of early death from chronic diseases such as diabetes (25-27) and cardiovascular disease (28-30) as well as hormone-dependent cancers such as breast cancer, ovarian cancer, and prostate cancer. Exposure to PCBs has been associated with numerous sex-hormone-related chronic diseases such as breast cancer (31), prostate cancer (32), type 2 diabetes (33,34), and coronary heart disease (35,36), which could be mediated at least in part by the actions of PCBs on sex hormones.

The purpose of this study is to investigate the associations of PCBs with testosterone and testosterone binding by SHBG, in a cohort of Great Lakes fish consumers.

4.2. Methods

4.2.1 Cohort Background

This study used data from the Great Lakes Sport-caught Fish Consumer cohort described in the previous chapter. Detailed description of participant selection is described elsewhere (3,37-39). Briefly, 4,206 US residents living near the Great Lakes were invited to participate in the original 1994 telephone survey in focused on fish consumption habits and health history. In 1994/1995, a stratified subsample (age/sex/region) of 619 mostly frequent consumers of Great Lakes Sport-caught fish participated in a follow-up study for which approximately 30 mL of blood was donated for analysis of PCBs and DDE (38). In a follow-up study of the 619 participants who donated blood in at baseline, 207 donated blood again from 2001-2003. A second follow-up study in 2003 included a questionnaire returned by 1788 of the original cohort, of which 515 volunteered to donate blood samples and completed an additional survey in 2004/2005 (37). For this study, the analytic sample was restricted to male participants who had at

least one measure of serum POPs, at least one measure of serum hormones, did not report diagnosis of thyroid disease or thyroid cancer, and had not recently taken thyroid hormone modifying drugs, steroid hormone modifying drugs, or corticosteroids.

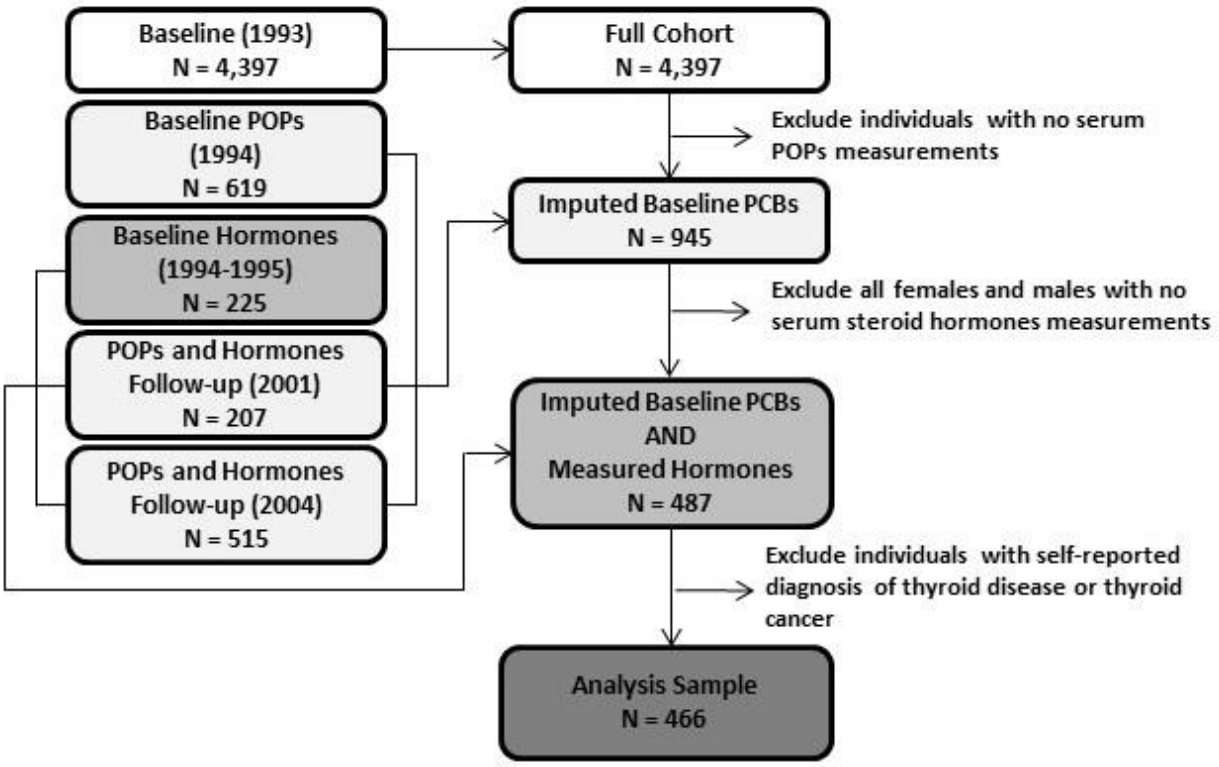

Figure 3. Study timeline and analytic sample inclusion criteria

4.2.2 Organochlorine Measurement

Samples were collected in red-top vacutainer tubes and the serum component was separated from remaining whole blood components. Serum samples were then stored at -20°C until analysis. Sera were analyzed for DDE and 89 PCB congeners using electron capture capillary column gas chromatography.

In 1994-1995, analysis efforts were split between the Wisconsin State Laboratory of Hygiene (Madison, WI) and the Michigan Department of Public Health Laboratory (Lansing, MI) while the latter two analyses were conducted entirely in Wisconsin. Concentrations of DDE and the 89 PCB congeners were quantified and represented by a total of 62 peaks using gas chromatography with electron capture detection (38) or mass spectrometry (39). Due to slight differences in analytic technique and instrumentation between laboratories and over time, only 18 PCB congeners represented by 13 unique peaks could be consistently quantified (74, 99, 118, 146, 180, 194, 201, 206, 132/153, 138/163, 170/190, 182/187, and 196/203) (40). On average, recovery was 97% for DDE and 81–94% for di- to hexaPCB congeners (37).

Analytes below the method detection limit (MDL) were imputed using censored maximum likelihood estimation (41) in R (42). Briefly, observations below the MDL were randomly assigned values between 0 and the respective MDL following a log-linear distribution of observations above the limit. For statistical analyses, PCB congeners were summed over all congeners and grouped according to their proposed metabolic pathways (43) for the following four PCB groupings: (1) Total PCBs, (2) MC-type PCBs, (3) PB-type PCBs, and (4) Mixed-type PCBs (Table I). DDE was analyzed as the sum of all isoforms.

4.2.3 Hormone Measurement

All samples were analyzed for serum concentrations of hormones at the Immunoassay Core Facility Laboratory of the Robert H. Lurie Comprehensive Cancer Center of Northwestern University (Chicago, IL). Methods for hormone measurements are described in detail elsewhere (3,37).

For samples collected during 1994/1995, steroid hormones were measured in a subsample of 225 non-diabetic male and female participants with at least 3.5mL of serum sample volume leftover from PCB/DDE analysis. Serum concentrations of SHBG were measured using the Delphia system, a solid phase two-site time-resolved fluoroimmunometric assay. The interassay coefficient of variation (CV) for SHBG was 6% and the intraassay CV was 8% (3). Serum concentrations of total testosterone were measured using coated tube assay, which cross-reacted with androstenedione/androstenediol at <1% and DHT at 5.8%. The interassay CV for total testosterone was 4.9% and the intraassay CV was 5.7% (3). SHBG-bound testosterone was measured directly using methods developed by Bonfrer et al. (44) and described in detail by Turyk et al. (37). The interassay CV for SHBG-bound testosterone was 8.2% and the intraassay CV was 10.4% (3). Free testosterone was also measured directly using methods described in detail by Persky et al. (3). The intraassay CV for free testosterone was 7.1% (3).

For samples collected during 2004-2005, serum concentrations of total testosterone, SHBG, and SHBG-bound testosterone were measured using the same methods described above for 1994/1995. The interassay / intraassay CVs were 17% / 6.6% for total testosterone, 15.7% / 6.6% for SHBG, and 15.7% / 6.6% for SHBG-bound testosterone (37). In addition, 93 stored samples collected during 2001-2003 from males and females were analyzed for steroid hormones along with the 354 samples collected in 1994/1995, using the same methods and quality control.

However, due to prolonged storage of samples from 2001-2003 and multiple freeze/thaw cycles, concentrations of SHBG and SHBG-bound testosterone could not be ascertained in these samples. Free testosterone was not measured directly in either follow-up study.

Due to inconsistencies in measurement between the three studies of the free and bound fractions of testosterone, the free and SHBG-bound fractions of testosterone were also estimated using methods described by Vermeulen et al. (45). Correlation between observed and estimated SHBG-bound testosterone was modest ($n = 344$; $r = 0.136$; $p = 0.0116$), but correlation between observed and estimated free testosterone was poor ($n = 164$; $r = 0.038$; $p = 0.6301$). To maintain consistency of measurement methods across studies, the estimated fractions of both SHBG-bound testosterone and free testosterone were used in all statistical analyses.

4.2.4 Covariate Ascertainment Methods

Demographic characteristics such as age, sex, body mass index (BMI), and education were reported at baseline. Race/ethnicity was excluded from the analysis since 99% of the study sample was non-Hispanic white. Income was excluded from the analysis due to a high proportion of missing values, likely missing not at random. BMI and smoking status were also reported at each comprehensive health follow-up questionnaire and each blood draw supplementary questionnaire. Smoking status was not reported at baseline, but smoking status at baseline was reported retrospectively in the 2010 comprehensive health questionnaire. Serum cholesterol and triglycerides were measured using serum samples collected from blood draws in 1996, 2001-2003 (Meriter Laboratories; Madison, WI), and 2004-2005 (Quest Diagnostics; Auburn Hills, MI; Wood Dale, IL). Total lipids were calculated using the following equation (46):

$$\text{Equation 2.}\quad \text{Total Lipids} = (2.27 * \text{Total Cholesterol}) + \text{Triglycerides} + 62.3$$

4.2.5 Statistical Analysis

Missing values for all POPs and covariates were imputed using the multiple imputations by chained equations (MICE) package in R 3.2.0 (R Foundation for Statistical Computing, Vienna, Austria). Details on the imputation method can be seen in Appendix A.

All PCB classifications and sex hormone measures were log-normally distributed and therefore were natural log transformed to meet the assumptions of linear regression. Tertiles of PCB classifications confirmed a general log-linear dose-response of PCB groupings with log-hormones. The result of such transformations is a change in the interpretation of beta coefficients to be "the percent change in Y for each 1% increase in X" and all beta coefficients were multiplied by 50 to represent a 50% increase in PCBs.

Likelihood-based mixed models were used to estimate associations of testosterone and testosterone binding with DDE and PCB groupings over time. Specifically, ln(hormone variables) were regressed on fixed effects of ln(PCB grouping variables), adjusted for age (time-varying), age^2 (time-varying), BMI (time-varying), BMI^2 (time-varying), baseline education (less than HS, HS equivalent, some college), smoking status (never, former, current; time-varying), total lipids (continuous), total lipids^2 (continuous), and study (1996, 2002, 2004), with a G-side random subject effect with compound symmetry correlation structure. Effect modification by age was addressed by replacing continuous age variables with a 3-category factor variable (<40 years, 40-59 years, ≥60 years) along with an interaction between the age factor variable and the relevant POP exposure variable. To account for testosterone feedback regulation of SHBG, a sensitivity analysis was included where models of PCBs on SHBG were additionally adjusted for log-transformed total testosterone.

Since diabetes was an exclusion criterion for the hormone measurements of the 1996 samples, and both diabetes and liver disease may potentially be bidirectional mediators and confounders of the associations of DDE and PCBs with sex hormones and sex hormone binding, a sensitivity analysis was conducted to restrict to participants who had never reported a diagnosis of diabetes or taking anti-diabetes medication and never reported diagnosis of liver disease. Additionally, a sensitivity analysis was conducted to trim the distribution at the 1st and 99th percentiles of the full analytic sample, deleting all observations at values below the 1st and above the 99th percentiles of total testosterone and SHBG.

This study was approved by the institutional review boards (IRB) for human studies at the University of Illinois in Chicago and at the University of Wisconsin Madison.

4.3. Results

There were 466 participants in the overall study sample (Figure 1), which consisted of primarily non-Hispanic white (98%) males (100%) between the ages of 40 and 60 years (68%). At baseline, most subjects were overweight or obese (81%), non-smokers (61%), with at least a high school diploma or equivalent (65%) (Table VI). Total number of participants with non-missing hormones at each time point displayed in Table VII.

Increasing age and BMI were associated with lower total testosterone and SHBG. SHBG-bound testosterone and free testosterone were consistently lower with increasing age and BMI, but means were not statistically different (Table VIII). Smoking, education, diabetes, and total lipids were not associated with any hormone endpoint (Table VIII). Factors associated with elevated DDE and PCBs include only increasing age and increasing BMI (Table IX, Table X).

TABLE VI. BASELINE CHARACTERISTICS OF THE STUDY SAMPLE [a]

Variable [b]	n (%)
Overall	466 (100.0)
Age Group	
20-39	79 (17.0)
40-59	316 (67.8)
60-75	71 (15.2)
BMI Category	
Norm/Under	91 (19.5)
Overweight	260 (55.8)
Obese	115 (24.7)
Smoking	
Non-smokers	287 (61.5)
Smokers	179 (38.5)
Education	
Less than HS	165 (35.4)
HS or Equiv.	148 (31.8)
Some College	153 (32.8)
Diabetes	
Never	391 (83.9)
Ever	75 (16.1)
Liver Disease	
Never	435 (93.3%)
Ever	31 (6.7%)
Total Lipids	
1st Tertile	139 (29.8)
2nd Tertile	152 (32.7)
3rd Tertile	175 (37.6)

[a] Freq. = Frequency; BMI = body mass index; HS = high school

[b] All characteristics were ascertained at baseline except for diabetes, which is defined as having been diagnosed at any point during follow-up.

TABLE VII. SAMPLE SIZE BY TIME POINT AND OVERALL [a]

Time Point	Total Testosterone	SHBG	SHBG-bound Testosterone	Free Testosterone
	N	*N*	*N*	*N*
Baseline	179	185	179	179
2001 Follow-up	49	0	0	0
2004 Follow-up	355	333	333	333
Any	464	454	450	450

[a] SHBG = steroid hormone binding globulin

TABLE VIII. GEOMETRIC MEANS LEVELS OF BASELINE CIRCULATING STEROID SEX HORMONES BY COHORT CHARACTERISTICS [a]

Variable [b]	Total Testosterone		SHBG		SHBG-bound Testosterone		Free Testosterone	
	Geomean (95% CI)	*p-value* [c]	*Geomean (95% CI)*	*p-value* [c]	*Geomean (95% CI)*	*p-value* [c]	*Geomean (95% CI)*	*p-value* [c]
Overall	352.8 (319.2, 389.9)	-	35.7 (33.0, 38.5)	-	185.3 (165.9, 206.9)	-	6.6 (6.0, 7.4)	-
Age Group								
20-39	422.6 (367.5, 486.1)	Ref.	29.8 (24.8, 35.9)	Ref.	198.6 (163.0, 242.0)	Ref.	9.1 (7.9, 10.4)	Ref.
40-59	362.3 (332.1, 395.3)	0.2752	35.3 (32.1, 38.8)	0.1179	189.4 (169.9, 211.0)	0.7633	6.8 (6.2, 7.5)	0.0543
60-75	275.2 (177.8, 425.9)	0.0135	43.4 (36.6, 51.4)	0.0049	161.7 (103.5, 252.7)	0.2878	4.5 (2.9, 7.0)	0.0001
BMI Category								
Norm/Under	386.8 (318.2, 470.2)	Ref.	44.3 (37.4, 52.4)	Ref.	226.3 (180.6, 283.6)	Ref.	6.4 (5.3, 7.6)	Ref.
Overweight	359.6 (318.0, 406.7)	0.5798	34.9 (31.5, 38.7)	0.0180	186.4 (162.2, 214.3)	0.1790	6.8 (6.0, 7.8)	0.6007
Obese	309.1 (234.9, 406.9)	0.1519	31.1 (26.7, 36.2)	0.0031	152.4 (115.4, 201.3)	0.0215	6.3 (4.7, 8.5)	0.9873
Smoking								
Non-smokers	337.8 (290.0, 393.5)	Ref.	36.1 (32.7, 39.8)	Ref.	179.7 (152.8, 211.3)	Ref.	6.3 (5.4, 7.4)	Ref.
Smokers	373.2 (331.1, 420.5)	0.3318	35.1 (31.0, 39.7)	0.7126	192.8 (166.7, 223.1)	0.5329	7.1 (6.2, 8.1)	0.2727
Education								
Less than HS	395.5 (345.8, 452.3)	Ref.	36.6 (31.9, 42.0)	Ref.	211.4 (178.8, 250.0)	Ref.	7.3 (6.4, 8.3)	Ref.
HS or Equiv.	315.7 (252.9, 394.2)	0.0746	35.6 (30.7, 41.3)	0.7688	164.4 (129.2, 209.1)	0.0712	5.9 (4.7, 7.5)	0.1138
Some College	354.3 (303.8, 413.1)	0.3764	34.8 (31.0, 39.2)	0.6022	184.7 (157.3, 216.9)	0.3250	6.8 (5.7, 8.1)	0.5726
Diabetes								
Never	352.5 (316.2, 393.0)	Ref.	36.2 (33.4, 39.3)	Ref.	186.3 (165.3, 210.0)	Ref.	6.6 (5.9, 7.3)	Ref.
Ever	356.1 (315.7, 401.6)	0.9558	30.3 (24.3, 37.9)	0.2001	174.4 (145.8, 208.6)	0.7439	7.4 (6.1, 8.9)	0.5388
Liver Disease								
Never	354.5 (322.7, 389.4)	Ref.	35.6 (32.9, 38.6)	Ref.	186.2 (167.4, 207.1)	Ref.	6.6 (6.0, 7.3)	Ref.
Ever	327.9 (132.1, 813.8)	0.7126	36.0 (27.4, 47.4)	0.9408	172.2 (69.8, 424.8)	0.7376	6.3 (2.5, 16.3)	0.8326
Total Lipids								
1st Tertile	377.4 (316.8, 449.6)	Ref.	35.6 (31.0, 40.9)	Ref.	198.5 (163.4, 241.1)	Ref.	7.1 (5.9, 8.5)	Ref.
2nd Tertile	325.4 (265.9, 398.1)	0.2422	34.5 (30.4, 39.0)	0.7337	169.6 (137.0, 209.9)	0.2610	6.2 (5.0, 7.7)	0.3039
3rd Tertile	360.6 (311.6, 417.3)	0.7181	36.9 (32.1, 42.4)	0.7173	190.6 (160.4, 226.5)	0.7716	6.6 (5.7, 7.8)	0.5914

[a] SHBG = steroid hormone binding globulin; Geomean = geometric mean; CI = confidence interval; Ref. = reference category; BMI = body mass index; HS = high school

[b] All characteristics were ascertained at baseline except for diabetes, which is defined as having been diagnosed at any point during follow-up.

[c] p-value for t-test of difference in log-mean from reference category

TABLE IX. GEOMETRIC MEANS LEVELS OF BASELINE PCBS BY COHORT CHARACTERISTICS [a]

Variable [b]	Total PCBs		MC-Type PCBs		PB-Type PCBs		Mixed-Type PCBs	
	Geomean (95% CI)	*p-value* [c]	*Geomean (95% CI)*	*p-value* [c]	*Geomean (95% CI)*	*p-value* [c]	*Geomean (95% CI)*	*p-value* [c]
Overall	4.60 (4.29, 4.95)	-	0.42 (0.37, 0.48)	-	2.64 (2.45, 2.83)	-	1.49 (1.37, 1.62)	-
Age Group								
20-39	3.76 (3.19, 4.43)	Ref.	0.34 (0.26, 0.44)	Ref.	2.14 (1.84, 2.49)	Ref.	1.24 (1.02, 1.51)	Ref.
40-59	4.76 (4.39, 5.17)	0.0071	0.44 (0.39, 0.49)	0.0519	2.73 (2.51, 2.97)	0.0033	1.54 (1.41, 1.69)	0.0280
60-75	4.97 (4.32, 5.71)	0.0094	0.45 (0.36, 0.57)	0.0518	2.85 (2.49, 3.27)	0.0060	1.58 (1.35, 1.86)	0.0384
BMI Category								
Norm/Under	4.19 (3.69, 4.76)	Ref.	0.36 (0.29, 0.45)	Ref.	2.44 (2.16, 2.75)	Ref.	1.33 (1.15, 1.55)	Ref.
Overweight	4.82 (4.43, 5.25)	0.0698	0.43 (0.38, 0.49)	0.1148	2.78 (2.55, 3.03)	0.0793	1.55 (1.41, 1.71)	0.0803
Obese	4.47 (3.94, 5.07)	0.4600	0.45 (0.36, 0.55)	0.1190	2.49 (2.20, 2.83)	0.7809	1.49 (1.29, 1.71)	0.2669
Smoking								
Non-smokers	4.68 (4.25, 5.15)	Ref.	0.44 (0.38, 0.51)	Ref.	2.67 (2.43, 2.94)	Ref.	1.50 (1.35, 1.68)	Ref.
Smokers	4.49 (4.08, 4.93)	0.5213	0.39 (0.33, 0.46)	0.1533	2.58 (2.34, 2.84)	0.5856	1.47 (1.32, 1.64)	0.7929
Education								
Less than HS	4.62 (4.17, 5.11)	Ref.	0.40 (0.34, 0.47)	Ref.	2.66 (2.41, 2.94)	Ref.	1.49 (1.32, 1.68)	Ref.
HS or Equiv.	4.64 (4.16, 5.18)	0.9370	0.44 (0.37, 0.53)	0.3649	2.61 (2.34, 2.91)	0.7748	1.53 (1.35, 1.74)	0.7238
Some College	4.56 (4.06, 5.11)	0.8600	0.42 (0.35, 0.50)	0.7771	2.64 (2.35, 2.96)	0.9163	1.45 (1.28, 1.65)	0.7616
Diabetes								
Never	4.55 (4.22, 4.90)	Ref.	0.41 (0.36, 0.46)	Ref.	2.62 (2.43, 2.82)	Ref.	1.47 (1.35, 1.60)	Ref.
Ever	4.91 (4.19, 5.75)	0.3553	0.49 (0.39, 0.62)	0.0956	2.74 (2.34, 3.21)	0.5694	1.62 (1.36, 1.94)	0.2701
Liver Disease								
Never	4.64 (4.30, 4.99)	Ref.	0.42 (0.37, 0.48)	Ref.	2.65 (2.46, 2.85)	Ref.	1.50 (1.38, 1.64)	Ref.
Ever	4.18 (3.34, 5.22)	0.3657	0.36 (0.26, 0.49)	0.2931	2.46 (1.97, 3.06)	0.4957	1.33 (1.06, 1.68)	0.3451
Total Lipids								
1st Tertile	4.31 (3.52, 5.28)	Ref.	0.40 (0.28, 0.56)	Ref.	2.48 (2.06, 2.97)	Ref.	1.39 (1.09, 1.78)	Ref.
2nd Tertile	4.58 (4.02, 5.22)	0.6164	0.43 (0.36, 0.51)	0.7122	2.61 (2.29, 2.98)	0.6405	1.48 (1.29, 1.70)	0.6353
3rd Tertile	4.93 (4.17, 5.83)	0.3807	0.44 (0.35, 0.55)	0.6487	2.83 (2.38, 3.36)	0.3399	1.60 (1.35, 1.89)	0.4270

[a] PCB = polychlorinated biphenyl; MC = 3-methylcholanthrene; PB = phenobarbital; Geomean = geometric mean; CI = confidence interval; Ref. = reference category; BMI = body mass index; HS = high school

[b] All characteristics were ascertained at baseline except for diabetes, which is defined as having been diagnosed at any point during follow-up.

[c] p-value for t-test of difference in log-mean from reference category

TABLE X. GEOMETRIC MEANS LEVELS OF BASELINE DDE BY COHORT CHARACTERISTICS [a]

Variable [b]	DDE	
	Geomean (95% CI)	*p-value* [c]
Overall	3.99 (3.26, 4.89)	-
Age Group		
20-39	2.41 (1.70, 3.40)	Ref.
40-59	4.27 (3.49, 5.24)	0.0002
60-75	5.18 (3.75, 7.16)	0.0004
BMI Category		
Norm/Under	3.21 (2.39, 4.30)	Ref.
Overweight	4.03 (3.23, 5.03)	0.1093
Obese	4.65 (3.53, 6.11)	0.0265
Smoking		
Non-smokers	4.01 (3.17, 5.06)	Ref.
Smokers	3.97 (3.12, 5.06)	0.9393
Education		
Less than HS	4.06 (3.17, 5.20)	Ref.
HS or Equiv.	4.20 (3.26, 5.41)	0.8016
Some College	3.74 (2.86, 4.89)	0.5835
Diabetes		
Never	3.78 (3.05, 4.67)	Ref.
Ever	5.35 (4.06, 7.04)	0.0149
Liver Disease		
Never	3.97 (3.22, 4.90)	Ref.
Ever	4.32 (2.84, 6.57)	0.7201
Total Lipids		
1st Tertile	3.73 (2.80, 4.98)	Ref.
2nd Tertile	3.87 (2.98, 5.03)	0.8459
3rd Tertile	4.39 (3.09, 6.23)	0.5097

[a] DDE = dichlorodiphenyldichloroethylene; Geomean = geometric mean; CI = confidence interval; Ref. = reference category; BMI = body mass index; HS = high school

[b] All characteristics were ascertained at baseline except for diabetes, which is defined as having been diagnosed at any point during follow-up.

[c] p-value for t-test of difference in log-mean from reference category

After adjusting for known and suspected confounders, there were no differences in mean total testosterone with a doubling of any PCB grouping. However, a borderline significant 13% reduction in mean SHBG was observed for a doubling in both PB-type PCBs (95% CI: -26.8, 1.2; p = 0.0722) and total PCBs (95% CI: -26.2, 1.1; p=0.0716). Similar results were seen for SHBG-bound testosterone for both PB-type PCBs (%chg. = -22.3; 95% CI: -52.4, 7.8; p=0.147) and total PCBs (%chg. = -20.0; 95% CI: -49.5, 9.4; p = 0.1828), though neither association reached statistical significance (Table XI).

Additionally, a doubling of MC-type PCBs, PB-type PCBs, and total PCBs were all associated with a borderline significant increase in free testosterone, with the strongest association in PB-type PCBs (%chg. = 8.4%; 95% CI: -1.8, 18.5; p = 0.1072). An 8% reduction in SHBG was also seen with a doubling of serum DDE, though results were not statistically significant (%chg. = -7.7; 95% CI: -17.9, 2.4; p = 0.1358). However, there was a strong and statistically significant inverse association of DDE with SHBG-bound testosterone (%chg. = 22.6; 95% CI: -42.6, -2.6; p = 0.0269). There were no associations of DDE with total testosterone or free testosterone (Table XI).

After removing all subjects who were diagnosed with liver disease at any point during the study, 435 of the original analytic sample remained. Estimates of adjusted associations of PCBs with SHBG increased in magnitude to a 16% reduction in SHBG for each doubling of PB-type (95% CI = -31.4, -1.5) and total PCBs (95% CI: -30.4, -1.3) with p-values reaching statistical significance for PB-type (p = 0.0306) and total PCBs (p = 0.0329). Similar results were seen for mixed-type PCBs to a lesser extent. Inverse associations of POPs with SHBG-bound testosterone observed in the model including all participants remained unchanged after excluding participants with liver disease (Table XI).

TABLE XI. MODEL-ADJUSTED MEAN PERCENT DIFFERENCE IN HORMONES WITH DOUBLING OF SERUM PCBS AND DDE [a]

Hormone Endpoint	PCB Grouping	All Observations (n = 466)		No Liver Disease (n = 435)		No Diabetes/Liver Disease (n = 365)		No Diabetes/Liver Disease and Trimmed at Extremes (n = 344)	
		% Chg (95% CI) [b]	*p-value* [c]	*% Chg (95% CI)* [b]	*p-value* [c]	*% Chg (95% CI)* [b]	*p-value* [c]	*% Chg (95% CI)* [b]	*p-value* [c]
Total Testosterone	Total PCBs	1.7 (-5.4, 8.7)	0.6417	1.0 (-6.3, 8.2)	0.793	2.0 (-6.3, 10.3)	0.6358	-2.3 (-9.0, 4.5)	0.5129
	MC-type PCBs	0.1 (-5.5, 5.6)	0.9825	-0.7 (-6.3, 4.9)	0.8054	-1.0 (-7.4, 5.5)	0.7635	-2.0 (-7.3, 3.2)	0.4453
	PB-type PCBs	2.3 (-4.9, 9.6)	0.5308	1.7 (-5.7, 9.1)	0.6561	2.9 (-5.6, 11.3)	0.5083	-1.9 (-8.8, 5.1)	0.5961
	Mixed-type PCBs	0.5 (-6.1, 7.2)	0.8742	0.4 (-6.4, 7.1)	0.918	1.4 (-6.3, 9.1)	0.718	-2.9 (-9.2, 3.4)	0.3630
	DDE	-2.5 (-7.6, 2.6)	0.3381	-1.1 (-6.3, 4.0)	0.665	-0.2 (-6.0, 5.5)	0.9333	-2.7 (-7.5, 2.2)	0.2828
SHBG	Total PCBs	-12.5 (-26.2, 1.1)	0.0716	-15.9 (-30.4, -1.3)	0.0329	-15.0 (-28.5, -1.4)	0.0309	-10.4 (-20.5, -0.4)	0.0423
	MC-type PCBs	-8.4 (-19.0, 2.3)	0.1243	-9.9 (-21.1, 1.4)	0.0865	-9.5 (-20.2, 1.1)	0.0785	-13.0 (-20.7, -5.3)	0.0009
	PB-type PCBs	-12.8 (-26.8, 1.2)	0.0722	-16.5 (-31.4, -1.5)	0.0306	-15.6 (-29.5, -1.8)	0.0271	-9.5 (-19.8, 0.8)	0.0703
	Mixed-type PCBs	-10.9 (-23.7, 1.9)	0.0958	-13.6 (-27.1, 0.0)	0.0502	-12.1 (-24.7, 0.6)	0.0619	-8.1 (-17.5, 1.3)	0.0925
	DDE	-7.7 (-17.9, 2.4)	0.1358	-8.0 (-18.7, 2.7)	0.1404	-7.4 (-17.1, 2.3)	0.1359	-4.3 (-11.6, 2.9)	0.2423
SHBG (Adj. Total T) [d]	Total PCBs	-13.8 (-27.5, -0.2)	0.0467	-17.1 (-31.6, -2.5)	0.0216	-16.6 (-30.2, -2.9)	0.0175	-9.7 (-19.5, 0.1)	0.0519
	MC-type PCBs	-9.4 (-20.0, 1.2)	0.0836	-10.6 (-21.9, 0.6)	0.0635	-10.5 (-21.1, 0.2)	0.0550	-12.4 (-19.9, -4.8)	0.0013
	PB-type PCBs	-14.2 (-28.2, -0.2)	0.0461	-17.9 (-32.8, -2.9)	0.0192	-17.4 (-31.3, -3.4)	0.0147	-8.9 (-19.0, 1.1)	0.0810
	Mixed-type PCBs	-11.6 (-24.4, 1.1)	0.0742	-14.3 (-27.8, -0.8)	0.0384	-13.2 (-25.9, -0.5)	0.0419	-7.1 (-16.3, 2.0)	0.1263
	DDE	-7.8 (-18.0, 2.3)	0.1305	-8.6 (-19.3, 2.1)	0.1144	-8.3 (-18.0, 1.5)	0.0979	-3.5 (-10.6, 3.5)	0.3257

[a] Chg = Change; CI = confidence interval; PCB = polychlorinated biphenyl; MC = 3-methylcholanthrene; PB = phenobarbital; DDE = Dichlorodiphenyldichloroethylene; SHBG = steroid hormone binding globulin; T = testosterone

[b] Estimates represent the percent change in hormone measure per 100% increase in PCBs, adjusted for age, BMI, education, smoking, serum lipids, and follow-up study

[c] p-value for model-based chi-square test of % change = 0

[d] Model additionally adjusted for total testosterone

TABLE XI (CONTINUED). MODEL-ADJUSTED MEAN PERCENT DIFFERENCE IN HORMONES WITH DOUBLING OF SERUM PCBS AND DDE [a]

Hormone Endpoint	PCB Grouping	All Observations (n = 466)		No Liver Disease (n = 435)		No Diabetes/Liver Disease (n = 365)		No Diabetes/Liver Disease and Trimmed at Extremes (n = 344)	
		% Chg (95% CI) [b]	*p-value* [c]	*% Chg (95% CI)* [b]	*p-value* [c]	*% Chg (95% CI)* [b]	*p-value* [c]	*% Chg (95% CI)* [b]	*p-value* [c]
SHBG-bound Testosterone	Total PCBs	-20.0 (-49.5, 9.4)	0.1828	-23.2 (-54.6, 8.2)	0.1468	-15.6 (-44.6, 13.5)	0.2939	-6.7 (-14.7, 1.4)	0.1035
	MC-type PCBs	-1.2 (-21.0, 18.7)	0.9095	-1.8 (-23.2, 19.5)	0.8675	4.9 (-15.9, 25.7)	0.6444	-5.8 (-12.0, 0.4)	0.0646
	PB-type PCBs	-22.3 (-52.4, 7.8)	0.1470	-25.9 (-58.1, 6.2)	0.114	-18.1 (-47.7, 11.6)	0.2328	-6.2 (-14.4, 2.1)	0.1423
	Mixed-type PCBs	-15.9 (-42.9, 11.0)	0.2462	-18.3 (-47.0, 10.3)	0.2099	-12.7 (-39.4, 13.9)	0.3485	-6.7 (-14.2, 0.8)	0.0789
	DDE	-22.6 (-42.6, -2.6)	0.0269	-22.9 (-43.9, -1.9)	0.0327	-20.0 (-39.6, -0.4)	0.0453	-4.2 (-9.9, 1.6)	0.1561
Free Testosterone	Total PCBs	8.1 (-1.8, 18.0)	0.1107	9.1 (-1.0, 19.2)	0.0763	11.1 (-0.3, 22.4)	0.0558	4.3 (-5.3, 14.0)	0.3768
	MC-type PCBs	7.6 (-0.3, 15.4)	0.0582	7.9 (0.0, 15.8)	0.0498	9.0 (0.1, 17.9)	0.0483	7.5 (0.0, 15.0)	0.0488
	PB-type PCBs	8.4 (-1.8, 18.5)	0.1072	9.7 (-0.6, 20.1)	0.0659	11.6 (0.0, 23.2)	0.0494	4.0 (-5.8, 13.9)	0.4209
	Mixed-type PCBs	5.3 (-4.0, 14.6)	0.2628	6.3 (-3.1, 15.7)	0.1902	7.9 (-2.6, 18.4)	0.1419	1.6 (-7.3, 10.6)	0.7240
	DDE	1.1 (-6.3, 8.5)	0.7713	2.8 (-4.5, 10.2)	0.4544	3.3 (-4.7, 11.3)	0.4165	0.2 (-6.7, 7.2)	0.9462

[a] Chg = Change; CI = confidence interval; PCB = polychlorinated biphenyl; MC = 3-methylcholanthrene; PB = phenobarbital; DDE = Dichlorodiphenyldichloroethylene; SHBG = steroid hormone binding globulin; T = testosterone

[b] Estimates represent the percent change in hormone measure per 100% increase in PCBs, adjusted for age, BMI, education, smoking, serum lipids, and follow-up study

[c] p-value for model-based chi-square test of % change = 0

[d] Model additionally adjusted for total testosterone

However, removal of participants with liver disease increased the magnitudes of associations between PCB groupings and free testosterone, which showed a 10% increase in free testosterone with a doubling of PB-type PCBs (95% CI: -0.6, 20.1; p = 0.0659). Similar results were seen in total PCBs and MC-type PCBs while mixed-type PCBs were not associated with free testosterone. Associations of DDE with steroid hormone endpoints were unaffected by exclusion of subjects diagnosed with diabetes or liver disease (Table XI). After excluding participants with diabetes in addition to those with liver disease, associations of PCBs and DDE with SHBG and SHBG-bound testosterone remained largely unchanged, but associations of PCBs with free testosterone increased to 12% with a doubling of PB-type PCBs (95% CI: 0.0, 23.2; p = 0.0494). Similar results were seen for total PCBs and MC-type PCBs (Table XI).

Additional trimming of the distributions of total testosterone and SHBG at the 1st and 99th percentiles in above analysis excluding participant with diabetes or liver disease resulted in an analytic sample of 344. Estimates of most DDE and PCB associations with steroid hormone endpoints were attenuated toward the null. Specifically, associations of PB-type and total PCBs with SHBG were attenuated to a 9.5% decrease (95% CI: -19.8, 0.8) in SHBG for each doubling of PCBs, but these associations were only borderline significant (p = 0.0703). However, the association of MC-type PCBs with SHBG increased in magnitude to a 13% (95% CI: -20.7, -5.3) reduction and it did reach statistical significance (p = 0.0009). Associations of all PCB groupings with SHBG-bound testosterone were reduced to roughly 6.5% and all groupings reached statistical significance or borderline statistical significance. There were no associations of any PCB grouping with total or free testosterone after trimming the distributions of total testosterone and SHBG in this subsample (Table XI).

Results from the second model, where participants were excluded if they were diagnosed with liver disease at any point during the study (n = 435), were additionally stratified by age group (Table XII). After stratifying by age, PB-type PCBs were inversely associated with total testosterone in the youngest age group (<40 years), null in the middle age group (40-59 years), and positively associated with total testosterone in the oldest age group (≥60 years), but these associations were not statistically significant. Similar results were seen for total PCBs, but not for MC-type, mixed-type or DDE. In contrast, all PCB groupings were inversely associated with SHBG, but the associations became stronger (more negative) with increasing age. None of these associations were statistically significant after stratifying be age. PB-type and total PCBs were also positively associated with SHBG-bound testosterone in the youngest age group, inversely associated with SHBG-bound testosterone in the middle age group, and inversely associated at a stronger magnitude (more negative) with SHBG-bound testosterone in the oldest age group. Results from models of free testosterone were similar to those of total testosterone, with inverse associations in the youngest age group, increasing to positive associations in the oldest age group. but none of these associations were statistically significant.

4.4. Discussion

This study demonstrates associations of PCBs with SHBG as well as bound and free testosterone and DDE with SHBG bound testosterone, but no associations with total testosterone in a cohort of Great Lakes sport-caught fish consumers and locally matched non-consumer referents. However, results varied depending on the compound and the PCB enzyme induction properties, with the strongest associations consistently found for PB-type inducer PCBs, and the weakest with MC-type inducers or mixed inducers.

TABLE XII. MODEL-ADJUSTED MEAN PERCENT DIFFERENCE IN HORMONES WITH DOUBLING OF SERUM PCBS AND DDE BY AGE GROUP [a]

Hormone Endpoint	PCB Grouping	20 - 39 years (n = 76)		40-59 years (n = 292)		60-83 years (n = 67)	
		% Chg (95% CI) [b]	*p-value* [c]	*% Chg (95% CI)* [b]	*p-value* [c]	*% Chg (95% CI)* [b]	*p-value* [c]
Total Testosterone	Total PCBs	-9.0 (-28.8, 10.8)	0.3725	2.5 (-6.1, 11.2)	0.5635	3.8 (-8.1, 15.8)	0.5280
	MC-type PCBs	-4.3 (-21.0, 12.4)	0.6151	1.1 (-6.0, 8.3)	0.7531	-0.9 (-9.4, 7.5)	0.8265
	PB-type PCBs	-10.8 (-31.4, 9.7)	0.3006	2.8 (-6.0, 11.6)	0.5343	6.6 (-6.0, 19.1)	0.3048
	Mixed-type PCBs	-6.4 (-24.1, 11.4)	0.4816	2.1 (-6.1, 10.3)	0.6207	0.5 (-10.2, 11.2)	0.9272
	DDE	-8.4 (-24.5, 7.8)	0.3095	2.8 (-3.5, 9.0)	0.3885	-10.7 (-19.0, -2.5)	0.0110
SHBG	Total PCBs	-3.8 (-39.2, 31.6)	0.8320	-7.1 (-23.9, 9.8)	0.4101	-15.5 (-38.6, 7.7)	0.1912
	MC-type PCBs	22.1 (-9.1, 53.3)	0.1645	-8.0 (-21.6, 5.5)	0.2429	-10.7 (-26.6, 5.2)	0.1880
	PB-type PCBs	-7.9 (-44.6, 28.7)	0.6721	-6.0 (-23.3, 11.2)	0.4915	-15.9 (-40.5, 8.6)	0.2034
	Mixed-type PCBs	-3.2 (-35.1, 28.6)	0.8426	-7.2 (-23.1, 8.6)	0.3713	-11.7 (-32.4, 9.0)	0.2678
	DDE	-9.5 (-38.4, 19.5)	0.5221	-1.5 (-13.9, 10.9)	0.8082	-7.8 (-24.5, 8.9)	0.3619
SHBG (Adj. Total T) [d]	Total PCBs	-13.8 (-27.5, -0.2)	0.0467	-17.1 (-31.6, -2.5)	0.0216	-16.6 (-30.2, -2.9)	0.0175
	MC-type PCBs	-9.4 (-20.0, 1.2)	0.0836	-10.6 (-21.9, 0.6)	0.0635	-10.5 (-21.1, 0.2)	0.0550
	PB-type PCBs	-14.2 (-28.2, -0.2)	0.0461	-17.9 (-32.8, -2.9)	0.0192	-17.4 (-31.3, -3.4)	0.0147
	Mixed-type PCBs	-11.6 (-24.4, 1.1)	0.0742	-14.3 (-27.8, -0.8)	0.0384	-13.2 (-25.9, -0.5)	0.0419
	DDE	-7.8 (-18.0, 2.3)	0.1305	-8.6 (-19.3, 2.1)	0.1144	-8.3 (-18.0, 1.5)	0.0979

[a] Chg = Change; CI = confidence interval; PCB = polychlorinated biphenyl; MC = 3-methylcholanthrene; PB = phenobarbital; DDE = Dichlorodiphenyldichloroethylene; SHBG = steroid hormone binding globulin

[b] Estimates represent the percent change in hormone measure per 100% increase in PCBs, adjusted for BMI, education, smoking, serum lipids, and follow-up study. Models additionally include categorical age group and an age group * ln-POP interaction term. Model excludes all participants reporting liver disease at any point during the study.

[c] p-value for model-based chi-square test of % change = 0

[d] Model additionally adjusted for total testosterone

TABLE XII (CONTINUED). MODEL-ADJUSTED MEAN PERCENT DIFFERENCE IN HORMONES WITH DOUBLING OF SERUM PCBS AND DDE BY AGE GROUP [a]

Hormone Endpoint	PCB Grouping	20 - 39 years (n = 76)		40-59 years (n = 292)		60-83 years (n = 67)	
		% Chg (95% CI) [b]	*p-value* [c]	*% Chg (95% CI)* [b]	*p-value* [c]	*% Chg (95% CI)* [b]	*p-value* [c]
SHBG-bound Testosterone	Total PCBs	10.9 (-44.6, 66.4)	0.7003	-18.7 (-50.9, 13.6)	0.2562	-19.3 (-60.7, 22.1)	0.3609
	MC-type PCBs	39.5 (-10.4, 89.4)	0.1210	-6.4 (-29.6, 16.7)	0.5864	2.6 (-23.4, 28.7)	0.8428
	PB-type PCBs	9.7 (-48.8, 68.1)	0.7454	-19.3 (-52.1, 13.4)	0.2473	-23.2 (-67.4, 20.9)	0.3026
	Mixed-type PCBs	8.3 (-42.8, 59.4)	0.7502	-14.9 (-44.8, 15.0)	0.3287	-13.1 (-48.8, 22.6)	0.4720
	DDE	-6.8 (-48.1, 34.6)	0.7485	-13.6 (-36.7, 9.6)	0.2517	-22.9 (-48.5, 2.7)	0.0791
Free Testosterone	Total PCBs	-8.1 (-34.9, 18.6)	0.5504	5.5 (-6.9, 17.9)	0.3837	13.5 (-3.9, 30.9)	0.1281
	MC-type PCBs	-5.7 (-28.3, 16.8)	0.6181	6.9 (-3.3, 17.1)	0.1838	8.2 (-4.0, 20.4)	0.1894
	PB-type PCBs	-9.1 (-36.8, 18.6)	0.5196	4.9 (-7.8, 17.6)	0.4503	16.5 (-1.8, 34.8)	0.0768
	Mixed-type PCBs	-6.9 (-30.9, 17.2)	0.5749	4.8 (-7.0, 16.5)	0.4267	6.7 (-8.9, 22.3)	0.3982
	DDE	-7.2 (-29.2, 14.8)	0.5189	3.8 (-5.3, 12.9)	0.4095	-8.0 (-20.7, 4.7)	0.2151

[a] Chg = Change; CI = confidence interval; PCB = polychlorinated biphenyl; MC = 3-methylcholanthrene; PB = phenobarbital; DDE = Dichlorodiphenyldichloroethylene; SHBG = steroid hormone binding globulin

[b] Estimates represent the percent change in hormone measure per 100% increase in PCBs, adjusted for BMI, education, smoking, serum lipids, and follow-up study. Models additionally include categorical age group and an age group * ln-POP interaction term. Model excludes all participants reporting liver disease at any point during the study.

[c] p-value for model-based chi-square test of % change = 0

[d] Model additionally adjusted for total testosterone

Much of the toxicological literature around PCBs has focused on estrogen receptor binding (i.e. estrogenicity) as a plausible mechanism of action for PCB affecting sex hormones (6,7). However, more recent research has identified PCB congeners which may directly, or through their metabolites, influence synthesis of cytochrome P450 enzymes, which play major roles in sex hormone metabolism (43,47). Thus, it is important to highlight the strong overlap between congeners included in estrogenicity-based PCB groupings and CYP-based PCB groupings to make comparisons between this study and studies conducted in the past. Specifically, the estrogenic and the anti-estrogenic PCB groups developed by Wolff et al. contains many of the same congeners as the PB-type inducer and MC type inducer PCB groups developed by Warner et al., respectively.

After including excluding participants who had been diagnosed with liver disease at any point during the study, associations of all PCB groupings with SHBG increased in magnitude for PB-type and total PCBs, reaching statistical significance. Similar results were seen for associations with free testosterone with magnitudes reaching 9.7% for each doubling of PB-type PCBs. This suggests that liver disease may play an important role in the associations of PCBs with circulating sex hormones, but the role may be complex. Liver disease may be caused at least in part, by exposure to PCBs, but it can also be related to older age and increased adiposity, both strong confounders of the associations of PCBs with sex hormones. Since liver disease has the potential to influence the main associations through multiple roles/pathways, it was important to exclude participants with liver disease to reach a more reliable estimate.

After additionally excluding participants who had been diagnosed with diabetes at any point during the study, all associations of all PCB groupings with circulating sex hormones remained the same, suggesting that diabetes did not play a significant role in the associations of

PCBs and DDE with circulating sex hormones. Trimming of total testosterone and SHBG at the 1st and 99th percentiles resulted in attenuated magnitudes of the associations, but results were consistently precise and at least borderline significant for all PCB groupings with SHBG and SHBG-bound testosterone. However, there were no longer any positive associations of PCB groupings with free testosterone, and associations of PCB groupings with total testosterone remained null. It was unclear whether participants in the highest and lowest 1% of distributions of total testosterone and SHBG were extreme outliers or were within the expected distributions. Since associations did not drastically change after trimming at the extremes, or with exclusion of participants with diabetes, it was concluded that the dataset excluding participants with liver disease is most reliable.

Most testosterone in the blood is bound to SHBG and albumin with only small portions circulating freely. The portion of testosterone bound to SHBG is not available for activation of androgen receptors while the free portions are entirely available. Free portions of testosterone are maintained within a narrow hormetic window through a feedback loop where changes in total and free testosterone regulate synthesis of SHBG to increase or decrease bioavailability of testosterone. In participants without liver disease, inverse associations of PCBs with SHBG (-16.5% for each doubling of PB-type PCBs, p=0.03) and SHBG-bound testosterone (-17.9%, p=0.02), paired with associations in the opposite direction for free testosterone (9.7%, p=0.07), are consistent with a PCB-induced inhibition of SHBG synthesis resulting in reduced binding of total testosterone to SHBG and leaving a larger proportion of testosterone circulating freely in the blood. We found a similar significant inverse association of DDE with SHBG-bound testosterone (-22.9%, p=.0.3), but limited evidence for an impact of DDE on SHBG (-8%, p=0.14) and free testosterone (2.8%, p=0.45). The findings for DDE are less suggestive of an

inhibition of SHBG synthesis than those for PCB but could support interference of DDE with the binding of testosterone to SHBG. However, since DDE and PCBs are highly correlated in this population (data not shown), we did not include both exposure in same model and thus there may be residual confounding in the above associations.

Highly correlated environmental exposure is an analytic obstacle for which many new methodologies have been explored. However, we were unable address high correlation between PCBs and DDE in the regression analyses due to limitations in current analytic methods. To the best of our knowledge, there has been no study demonstrating an inverse association of serum DDE with testosterone binding (i.e. SHBG-bound or free testosterone) and several studies have demonstrated lack of evidence for this association (3,19,48,49). Furthermore, several studies have also demonstrated strong correlation between serum concentrations of DDE and PCBs (Rugnell-Hydbom 2005, Goncharov 2009, Haugen 2011, Persky 2011). While the previous literature has similarly shown lack of evidence for relationships between DDE and testosterone binding, these studies are also subject to the same potential bias introduced by high correlation between PCBs and DDE. After stratifying participants without liver disease by age, PB-type and total PCBs were most strongly associated inversely associated with total testosterone in the youngest age group, but these associations increased to slightly positive in the middle age group and moderately positive in the oldest age group. While results were strongest for PB-type PCBs, they were consistent across all PCB groupings. However, due to stratification by age groups of an already small sample, we did not have the power in any age group to detect significant differences in total testosterone with increasing PCBs. Effect modification by age was also seen in associations of PCBs with SHBG. All PCB groupings were inversely associated with SHBG, but associations were the strongest in the oldest age group. However, after adjusting for the total

testosterone, PCB associations with SHBG were consistently strong across all age groups. This may indicate that changes in total testosterone with increasing PCBs may to some extent be influencing changes in SHBG. While this would not fully explain the consistent inverse associations of PCBs with SHBG in all age groups, it may explain some of the age-related differences in association of PCBs with SHBG.

The literature around PCBs, testosterone, and SHBG provides inconsistent results and high variability with respect to study populations, biomarker measurement, and statistical methodology. In a study of young Swedish males (age 18-21), after adjusting for BMI, duration of abstinence, and smoking, the authors found no association between PCB-153 and total testosterone but the authors did find a significant increase in SHBG with increasing PCB-153 (21). In a pooled study of 749 males from Greenland (n=258), Warsaw (m=113), Sweden (n=184), and Kharkiv (n=194), there were no association of PCB-153 with free testosterone overall or in any of the 4 individual studies, but a significant positive association of PCB-153 with SHBG was observed in the Kharkiv study [β=3.60 (1.74, 5.46)] (49). Finally, in a study of 63 male workers at an electrical capacitor plant in LaSalle, IL (age 35+ yrs.), Persky et al. (23) found marginally significant ($0.5 \leq p < 0.10$) positive correlation between estrogenic PCBs and total testosterone (r=0.03) and a significant positive correlation between estrogenic PCBs and DHEAS (r=0.13). These few examples demonstrate results that conflict with the results of our study, but most of the literature demonstrates null or inverse associations of PCBs with SHBG, testosterone, and testosterone binding.

In a study of 257 adult (age 18-95 yrs.) male Akwesanse Mohawk Native Americans, total testosterone was significantly lower among males in the highest tertile of serum total PCBs concentration compared to the lowest (OR=0.17; CI not reported) after adjusting for age, BMI,

total lipids, HCB, DDE, and Mirex, but testosterone binding to SHBG was not assessed (20). This study also found significant inverse associations of total testosterone with 4 of 5 PCB groupings (mon-ortho, di-ortho, tri-tetra-ortho, dioxin-like TEQs) and 4 of 9 individual PCB congeners (PCB 74, PCB 99, PCB 153, PCB 206). A third study of 101 young (age 20-40 yrs.) Flemish males demonstrated a 7.1% decrease (-0.5%, -13.2%; p=0.04) in total testosterone and a 6.8% decrease in free testosterone (-0.4%, -12.7%; p=0.04) with a 2-fold increase in dioxin-like TEQs (CALUX-TEQs) (18). A study of 341 males (age 18-51 yrs.) attending a US infertility clinic demonstrated marginally significant ($p<0.10$) inverse associations of anti-estrogenic PCBs with both SHBG and total testosterone, but not free testosterone, after adjusting for age, BMI, and total lipids, but since raw betas were presented with different transformations used for each variable in each model, estimates are not do not have a direct clinical interpretation (19). A study of 196 middle-aged and elderly Swedish males (age 48-82 yrs.) found no significant relationship between PCBs and total testosterone or SHBG (22). In a study of 277 healthy, non-obese, middle-aged males (age 45-69 yrs.) from the French West Indies, a 2-fold increase in PCB 153 was associated with a 5.4% increase (1.0%, 9.8%) in androstenedione, a metabolic precursor to testosterone (not to be confused with 3α-androstanediol, a metabolite of testosterone), but no associations of PCBs with total, free, or SHBG-bound testosterone (50).

Two cross-sectional studies on steroid hormones and PCB and DDE exposure have previously been conducted on the Great Lakes Sport-caught Fish Consumer cohort; one at baseline (1996) and the other at the second follow-up study in 2004. However, the data has not been pooled, accounting for variability over time, nor has data from the first follow-up study in 2002 been reported. In a study of 179 adult males free of diagnosed diabetes (mean age = 50 yrs.) from the Great Lakes Sport-Caught Fish Consumer cohort at baseline (1996), serum total

PCBs were significantly inversely associated with both total testosterone (r=-0.10) and percent SHBG-bound testosterone (r=-0.23), but no associations with either SHBG or percent free testosterone, after adjusting for age, BMI, medications, years eating Great Lakes fish, and total GL fish meals (3). However, a study in diabetes-free participants from the 2004 follow-up study of the Great Lakes Sport-caught Fish Consumer Cohort found no association of total PCBs or total TEQs with total testosterone (r=0.02; p=0.87) or SHBG (-0.09; p=0.63), but did find a similar inverse association of total PCBs with SHBG-bound testosterone (r=-0.29; p=0.04) (2).

The inverse associations of total PCBs with SHBG-bound testosterone were consistent with the current study, and both previous cross-sectional studies reported a non-significant inverse association of PCBs with SHBG as well. However, the inverse association of total PCBs with total testosterone and inconsistent association of PCBs with free testosterone in these two studies does not agree with the results of the current study. It is important to note that while these studies use many of the same participants and the same data, the analytic methods differ substantially between all three, but particularly in the current study. For example, the current study uses fully conditional specification of multiple imputations by chained equations for all missing PCBs. This is a major difference from the previous two studies, which used list-wise deletion of missing data, leaving only participants with non-missing data on PCBs. This has implications toward selection bias in the analytic sample, as listwise deletion assumes missing completely at random (MCAR), while multiple imputation and likelihood-based linear mixed models assume missing at random (MAR). Furthermore, this study used estimation of SHBG-bound and free testosterone while both previous studies used assays to directly measure the SHBG-bound and free fractions. Finally, this study incorporated additional data from

participants who had hormones measured in 2002, which were not used in either of the previous studies.

As with any observational study, this study has its limitations. The most notable limitation in this study was that limited resources prevented us from having measurements of organochlorines and steroid hormones in the entire cohort, which substantially restricted the sample size of our study. Furthermore, the specific hormone endpoints measured varied between baseline and follow-up studies due to limited resources as well as changes in the focus of the study over time. Since serum organochlorines were not measured in the same participants at all follow-ups, there was a large proportion of missing data. To address this, we imputed all missing data using fully conditional specification of multiple imputations by chained equations. While this method allowed us to recover observed data from participants that would otherwise have been deleted from the analysis due to missing data, it also introduced additional variability and imprecision in variables with high missingness and poor prediction.

As noted earlier in this section, a ubiquitous limitation in much of the epidemiological literature focused on environmental pollutants is the inability to isolate independent associations of each compound or congener with steroid hormone endpoints. While this was addressed within PCB congeners by grouping PCBs according to similarities in the metabolic CYP enzymes that they induce, we were not able to independently assess relationships between DDE and steroid hormones due to high correlation between DDE and PCBs.

As with all longitudinal cohorts, many participants were lost to follow-up either due to either voluntary non-participation or death. Charter boat captains had a vested interest in the study and were more likely to respond to follow-up surveys and offer follow-up blood samples than referents. As a result, we observed more frequent drop out in referents compared to charter

boat captains (34). Charter boat captains also eat Great Lakes sport-caught fish more frequently than referents resulting in elevated serum POPs relative to referents (51).

Despite its limitations, this study benefits from a longitudinal design spanning 8 years of follow-up as well as multiple measurements of PCB exposure and steroid hormone outcome biomarkers over the course of the study.

Results from our study support an inverse association of PCBs with SHBG and testosterone binding. While much of the exposure data was missing, the computationally complex imputation methods allowed us to recover missing information and include participants with missing data that would otherwise have been excluded from the analysis, which greatly increased the statistical power of the study. Finally, with multiple comprehensive health and fish consumption questionnaires, we were able to adjust for many known confounders of these associations resulting in a maximally precise and minimally biased estimate of these associations. While these methods greatly increased statistical power, additional longitudinal studies will be necessary to confirm these results.

4.5. Conclusions

This study demonstrated that exposure to PCBs may be associated with decreased SHBG and SHBG-bound testosterone, and with increased free testosterone, while DDE may be inversely associated with SHBG-bound testosterone. Associations were consistently stronger for PCB that were phenobarbital-type inducers compared with 3-methylcholanthrene inducers. Liver disease appeared to play an important role in these associations demonstrated by notably stronger associations after excluding participants with this disease state. Furthermore, it was not possible to estimate independent effects of DDE and PCBs in the presence of a simultaneous mixture of

exposures. Additional longitudinal studies are needed to confirm these results and future studies should focus on investigating other potential mechanisms of action as well a development of new statistical methods to independently assess relationships between steroid hormones and multiple highly correlated exposures.

Cited Literature

1. Knobeloch L, Turyk M, Imm P, Anderson H. Polychlorinated biphenyls in vacuum dust and blood of residents in 20 Wisconsin households. *Chemosphere*. 2012;86(7):735-740.

2. Turyk ME, Anderson HA, Freels S, et al. Associations of organochlorines with endogenous hormones in male Great Lakes fish consumers and nonconsumers. *Environ Res*. 2006;102(3):299-307.

3. Persky V, Turyk M, Anderson HA, et al. The effects of PCB exposure and fish consumption on endogenous hormones. *Environ Health Perspect*. 2001;109(12):1275-1283.

4. Hagmar L. Polychlorinated biphenyls and thyroid status in humans: A review. *Thyroid*. 2003;13(11):1021-1028.

5. Van den Berg M, Birnbaum LS, Denison M, et al. The 2005 world health organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. *Toxicol Sci*. 2006;93(2):223-241.

6. Wolff MS, Camann D, Gammon M, Stellman SD. Proposed PCB congener groupings for epidemiological studies. *Environ Health Perspect*. 1997;105(1):13-14.

7. Cooke PS, Sato T, Buchanan DL, Robertson L, Hansen L. Disruption of steroid hormone signaling by PCBs. *PCBs Recent Advances in Environmental Toxicology and Health Effects*. 2001:257-263.

8. Hansen LG. Stepping backward to improve assessment of PCB congener toxicities. *Environ Health Perspect*. 1998;106 Suppl 1:171-189.

9. Jansen HT, Cooke PS, Porcelli J, Liu T, Hansen LG. Estrogenic and antiestrogenic actions of PCBs in the female rat: In vitro and in vivo studies. *Reproductive Toxicology*. 1993;7(3):237-248.

10. Hany J, Lilienthal H, Sarasin A, et al. Developmental exposure of rats to a reconstituted PCB mixture or aroclor 1254: Effects on organ weights, aromatase activity, sex hormone levels, and sweet preference behavior. *Toxicol Appl Pharmacol*. 1999;158(3):231-243.

11. Negri-Cesi P, Colciago A, Pravettoni A, Casati L, Conti L, Celotti F. Sexual differentiation of the rodent hypothalamus: Hormonal and environmental influences. *J Steroid Biochem Mol Biol*. 2008;109(3-5):294-299.

12. Govarts E, Nieuwenhuijsen M, Schoeters G, et al. Birth weight and prenatal exposure to polychlorinated biphenyls (PCBs) and dichlorodiphenyldichloroethylene (DDE): A meta-analysis within 12 European birth cohorts. *Environ Health Perspect*. 2012;120(2):162-170.

13. Vafeiadi M, Agramunt S, Papadopoulou E, et al. In utero exposure to dioxins and dioxin-like compounds and anogenital distance in newborns and infants. *Environ Health Perspect*. 2013;121(1):125-130.

14. Neugebauer J, Wittsiepe J, Kasper-Sonnenberg M, Schöneck N, Schölmerich A, Wilhelm M. The influence of low level pre-and perinatal exposure to PCDD/fs, PCBs, and lead on attention performance and attention-related behavior among German school-aged children: Results from the Duisburg Birth Cohort Study. *Int J Hyg Environ Health*. 2015;218(1):153-162.

15. Winneke G, Ranft U, Wittsiepe J, et al. Behavioral sexual dimorphism in school-age children and early developmental exposure to dioxins and PCBs: A follow-up study of the Duisburg cohort. *Environ Health Perspect*. 2014;122(3):292-298.

16. Burns JS, Lee MM, Williams PL, et al. Associations of peripubertal serum dioxin and polychlorinated biphenyl concentrations with pubertal timing among Russian boys. *Environ Health Perspect*. 2016;124(11):1801-1807.

17. Denham M, Schell LM, Deane G, et al. Relationship of lead, mercury, Mirex, dichlorodiphenyldichloroethylene, hexachlorobenzene, and polychlorinated biphenyls to timing of menarche among Akwesasne Mohawk girls. *Pediatrics*. 2005;115(2):e127-34.

18. Dhooge W, van Larebeke N, Koppen G, et al. Serum dioxin-like activity is associated with reproductive parameters in young men from the general Flemish population. *Environ Health Perspect*. 2006;114(11):1670-1676.

19. Ferguson KK, Hauser R, Altshul L, Meeker JD. Serum concentrations of p, p′-DDE, HCB, PCBs and reproductive hormones among men of reproductive age. *Reproductive Toxicology*. 2012;34(3):429-435.

20. Goncharov A, Rej R, Negoita S, et al. Lower serum testosterone associated with elevated polychlorinated biphenyl concentrations in native American men. *Environ Health Perspect*. 2009;117(9):1454-1460.

21. Richthoff J, Rylander L, Jonsson BA, et al. Serum levels of 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153) in relation to markers of reproductive function in young males from the general Swedish population. *Environ Health Perspect*. 2003;111(4):409-413.

22. Rylander L, Wallin E, Jönssson BA, Stridsberg M, Erfurth EM, Hagmar L. Associations between CB-153 and p, p′-DDE and hormone levels in serum in middle-aged and elderly men. *Chemosphere*. 2006;65(3):375-381.

23. Persky V, Piorkowski J, Turyk M, et al. Polychlorinated biphenyl exposure, diabetes and endogenous hormones: A cross-sectional study in men previously employed at a capacitor manufacturing plant. *Environ Health*. 2012;11(1):57.

24. Feldman HA, Longcope C, Derby CA, et al. Age trends in the level of serum testosterone and other hormones in middle-aged men: Longitudinal results from the Massachusetts Male Aging Study. *The Journal of Clinical Endocrinology & Metabolism*. 2002;87(2):589-598.

25. Liu S, Sun Q. Sex differences, endogenous sex-hormone hormones, sex-hormone binding globulin, and exogenous disruptors in diabetes and related metabolic outcomes. *Journal of diabetes*. 2018;10(6):428-441.

26. Kim C, Halter JB. Endogenous sex hormones, metabolic syndrome, and diabetes in men and women. *Curr Cardiol Rep*. 2014;16(4):467.

27. Ding EL, Song Y, Malik VS, Liu S. Sex differences of endogenous sex hormones and risk of type 2 diabetes: A systematic review and meta-analysis. *JAMA*. 2006;295(11):1288-1299.

28. Clegg D, Hevener AL, Moreau KL, et al. Sex hormones and cardiometabolic health: Role of estrogen and estrogen receptors. *Endocrinology*. 2017;158(5):1095-1105.

29. Kloner RA, Carson C, Dobs A, Kopecky S, Mohler ER. Testosterone and cardiovascular disease. *J Am Coll Cardiol*. 2016;67(5):545-557.

30. El Khoudary SR, Shields KJ, Janssen I, et al. Cardiovascular fat, menopause, and sex hormones in women: The SWAN cardiovascular fat ancillary study. *The Journal of Clinical Endocrinology & Metabolism*. 2015;100(9):3304-3312.

31. Zhang J, Huang Y, Wang X, Lin K, Wu K. Environmental polychlorinated biphenyl exposure and breast cancer risk: A meta-analysis of observational studies. *PLoS One*. 2015;10(11):e0142513.

32. Prince MM, Hein MJ, Ruder AM, Waters MA, Laber PA, Whelan EA. Update: Cohort mortality study of workers highly exposed to polychlorinated biphenyls (PCBs) during the manufacture of electrical capacitors, 1940-1998. *Environ Health*. 2006;5:13.

33. Zong G, Valvi D, Coull B, et al. Persistent organic pollutants and risk of type 2 diabetes: A prospective investigation among middle-aged women in nurses' health study II. *Environ Int*. 2018;114:334-342.

34. Turyk M, Anderson H, Knobeloch L, Imm P, Persky V. Organochlorine exposure and incidence of diabetes in a cohort of Great Lakes sport fish consumers. *Environ Health Perspect*. 2009;117(7):1076-1082.

35. Bergkvist C, Berglund M, Glynn A, Wolk A, Åkesson A. Dietary exposure to polychlorinated biphenyls and risk of myocardial infarction–A population-based prospective cohort study. *Int J Cardiol*. 2015.

36. Blackowicz M, Persky V, Freels S, Anderson H, Turyk M. Cardiovascular disease incidence and exposure to persistent organic pollutant in Great Lakes fish consumers. . 2016.

37. Turyk ME, Persky VW, Imm P, Knobeloch L, Chatterton R, Anderson HA. Hormone disruption by PBDEs in adult male sport fish consumers. *Environ Health Perspect*. 2008;116(12):1635-1641.

38. Hanrahan LP, Falk C, Anderson HA, Draheim L, Kanarek MS, Olson J. Serum PCB and DDE levels of frequent Great Lakes sport fish consumers—a first look. *Environ Res*. 1999;80(2):S26-S37.

39. Anderson HA, Imm P, Knobeloch L, et al. Polybrominated diphenyl ethers (PBDE) in serum: Findings from a US cohort of consumers of sport-caught fish. *Chemosphere*. 2008;73(2):187-194.

40. Turyk M, Anderson H, Knobeloch L, Imm P, Persky V. Organochlorine exposure and incidence of diabetes in a cohort of Great Lakes sport fish consumers. *Environ Health Perspect*. 2009;117(7):1076-1082.

41. Jones RM, Stayner LT, Demirtas H. Multiple imputation for assessment of exposures to drinking water contaminants: Evaluation with the atrazine monitoring program. *Environ Res*. 2014;134:466-473.

42. R Developer Core Team. R: A language and environment for statistical computing. 2008.

43. Warner J, Osuch JR, Karmaus W, et al. Common classification schemes for PCB congeners and the gene expression of CYP17, CYP19, ESR1 and ESR2. *Sci Total Environ*. 2012;414:81-89.

44. Bonfrer J, Bruning P, Nooijen W. A simple method for the measurement of the steroid fraction bound to sex hormone binding globulin in serum. *J Steroid Biochem*. 1989;33(2):227-231.

45. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. *The Journal of Clinical Endocrinology & Metabolism*. 1999;84(10):3666-3672.

46. Bernert JT, Turner WE, Patterson DG, Needham LL. Calculation of serum "total lipid" concentrations for the adjustment of persistent organohalogen toxicant measurements in human samples. *Chemosphere*. 2007;68(5):824-831.

47. Uwimana E, Ruiz P, Li X, Lehmler H. Human cyp2a6, cyp2b6 and cyp2e1 atropselectively metabolize polychlorinated biphenyls to hydroxylated metabolites. *Environ Sci Technol*. 2019.

48. Giwercman AH, Rignell-Hydbom A, Toft G, et al. Reproductive hormone levels in men exposed to persistent organohalogen pollutants: A study of Inuit and three European cohorts. *Environ Health Perspect*. 2006;114(9):1348-1353.

49. Emeville E, Giton F, Giusti A, et al. Persistent organochlorine pollutants with endocrine activity and blood steroid hormone levels in middle-aged men. *PloS one*. 2013;8(6):e66460.

50. Hanrahan LP, Falk C, Anderson HA, et al. Serum PCB and DDE levels of frequent Great Lakes sport fish consumers—a first look. *Environ Res*. 1999;80(2):S26-S37.

5. CROSS-SECTIONAL ASSOCIATIONS OF PCBS WITH CIRCULATING SEX HORMONES: RESULTS FROM NHANES 1999-2002

5.1. Introduction

Polychlorinated biphenyls are a group of environmental pollutants consisting of 209 congeners that differ based on the number and position of chlorine atoms replacing hydrogen atoms on their two phenyl rings. PCBs were used extensively as lubricants and coolants in electrical equipment, such as high-line electrical capacitors, until US production was banned in 1978. Although PCBs are no longer manufactured in the US, PCBs continue to persist in the environment and in human tissues due to their resistance to degradation and long biological half-lives (1) and ongoing exposure from certain foods such as meat, dairy products, and fatty fish sourced from contaminated waters (2,3).

There is an extensive body of epidemiologic literature demonstrating the endocrine-disrupting effects of PCBs, but many of these studies are inconsistent with respect to both PCB exposure ascertainment and statistical methodology. Research on the endocrine disruption effects of PCBs has primarily been focused on thyroid hormones (4-6). Though less commonly studied, there has also been an interest in relationships between PCBs and steroid sex hormone disruption.

Steroid sex hormones are generally classified into two groups, androgens and estrogens, though a third class, progestogens, also plays an important role. Under normal conditions, sex steroid hormones remain in physiological balance in both males and females, and disruption of that balance can have short-term effects, such as acne, hirsutism, hair loss, hot flashes, menstrual disorder (females) and erectile dysfunction (males), as well as long-term effects, such as

accelerated atherosclerosis, increased risk of cardiovascular disease, and increased risk of early death from stroke, and myocardial infarction.

Aryl hydrocarbon receptor (AhR) activation by dioxins and dioxin-like PCBs has been well studied (7) and activation of the AhR has been suggested to have anti-androgenic effects (8,9) in males. Non-dioxin-like PCB congeners may act on other biochemical pathways either promoting or inhibiting production of estrogen through interactions with hormone receptors (8,9) and some PCB congeners may modify hormone metabolism through activation of enzymes that play key roles in sex hormone metabolism and homeostasis (10,11).

Polychlorinated biphenyls are metabolized by a group of enzymes called cytochrome P450s (CYP). Exposure to PCBs results in induction of CYPs to aid in metabolism and elimination of PCBs. Specifically, coplanar non-ortho substituted (dioxin-like) PCBs are strong MC-type inducers of metabolic enzymes CYP1A1, CYP1A2, and CYP1B1 while highly chlorinated non-coplanar ortho-substituted (non-dioxin-like) PCBs are strong PB-type inducers of metabolic enzymes CYP2B1, CYP2B2, Cp2A1, and CYP3A (12). Lower chlorinated mono-ortho-substituted PCBs can induce both MC-type and PB-type response and are referred to as mixed-type inducers (12). While these enzymes play a critical role in PCB metabolism and elimination, they are also involved in synthesis and metabolism of steroid sex hormones. Thus, induction of these enzymes has the potential to modify endogenous sex hormone profiles. Animal models have shown both estrogenic and anti-estrogenic PCB effects through abnormal development of sex organs (13,14) as well as sexual differentiation and behavior in the brain (15).

There is a growing body of literature around in-utero and early childhood exposure to PCBs and their relation to fetal development (16,17), early childhood development (18,19), and

sexual maturation (20,21). In addition to in-utero and childhood exposure to PCBs, exposure in adults has been inconsistently associated with numerous sex-hormone-related chronic diseases such as breast cancer (22), prostate cancer (23), type 2 diabetes (24,25), and coronary heart disease (26,27), which could be mediated at least in part by the actions of PCBs on sex hormones. However, epidemiologic evidence of the relationships between PCBs and circulating sex hormones in adults is sparse and inconsistent. For example, there are 4 previous studies that found an inverse association between PCBs and total testosterone (28-30). In contrast, another 3 studies that found no association between PCBs and total testosterone (4,31,32) and a fourth that found a positive association with a few congeners (33). However, these investigations measured different PCB congeners, employed different strategies for grouping congeners, controlled for different confounders, and infrequently examined modification by biological and demographic factors. Furthermore, the age distribution of participants varied across studies, which could also increase inconsistencies since steroid hormones are dramatically influenced by aging.

There is substantial evidence from toxicological studies that PCBs and dioxin-like chemicals influence endogenous hormones, and that sex hormones can affect risk of early death from chronic diseases such as diabetes (34-36) and cardiovascular disease (37-39) as well as hormone-dependent cancers such as breast cancer, ovarian cancer, and prostate cancer. However, the epidemiological data on the impact of chronic PCB exposure on sex hormones in males is sparse and inconsistent. The purpose of this study is to investigate the associations between PCB exposures and multiple sex hormone measures including steroid hormone-binding globulin (SHBG), testosterone, SHBG-bound testosterone, free testosterone, estradiol, SHBG-bound estradiol, free estradiol, and 3α-androstanediol glucuronide (3α-ADG) in a nationally representative sample of adult males in the U.S. We used exposure measures grouping

chemicals by common biochemical mechanisms to explore biological pathways and investigated modification by age, adiposity and diabetes status to identify potential subpopulations at increased risk.

5.2. Methods

5.2.1 Study Sample

The National Health and Nutrition Examination Survey (NHANES) is a nationally representative sample of the non-institutionalized US population gathering information from participant interviews, physical examinations, and laboratory tests. This study utilized the 1999-2000 and 2001-2002 cycles of NHANES as the analytic data set. In these two cycles, a one-third subsample of participants aged 12 years and older were selected for measurement of PCBs, dioxins, and furans. Males age 12 and older from the one-third PCB subsample were selected for measurement of circulating sex hormones from remaining stored serum samples. NHANES 1999-2004 was approved by the NCHS Institutional Review Board and all participants provided informed consent to participate in the survey (Protocol #98-12).

5.2.2 Data collection

Interviews, physical examinations, and laboratory blood draws were conducted by trained clinicians at the mobile examination centers (MEC). Information collected during the interview process includes age, race/ethnicity, education, smoking status, alcohol consumption, poverty to income ratio while body mass index (BMI) was assessed during the physical examination. Blood samples were collected at the MEC using methods described elsewhere (40). Briefly, non-fasting blood samples were collected in red-top Vacutainers, allowed to clot for 20-30 min, and

centrifuged for 10 minutes to isolate the serum fraction. Serum was then aliquoted and stored at -20°C until laboratory analysis at The National Center for Environmental Health.

5.2.3 Laboratory Methodology

Organochlorine compounds were measured using serum from a 1/3 subsample of the full MEC sample (Subsample C). Serum concentrations of 38 PCB congeners, 6 dioxins, and 10 furans (Table XIII) were analyzed using high-resolution gas chromatography/isotope-dilution high resolution mass spectrometry (HRGC/ID-HRMS). Analytes below the method limit of detection (MDL) were assigned a value of the MDL divided by the square root of 2. Due to strong correlations among organochlorines, concentrations of PCB congeners and dioxins/furans were summed using 5 techniques related to their proposed activity on biologic pathways. The 7 summation techniques are [1] total PCBs, [2] total TCDD TEQs (7), [3] MC-type inducers (10), [4] PB-type inducers (10), and [5] mixed-type inducers (10).

TABLE XIII. PCB GROUPINGS AND INCLUDED PCB CONGENERS [a]

PCB Grouping	PCB Congeners Included [b]
Total PCBs	PCBs: 28, 44, 49, 52, 66, 74, 81, 87, 99, 101, 105, 110, 118, 126, 128, 138, 146, 149, 151, 153, 156, 157, 167, 169, 170, 172, 177, 178, 180, 183, 187, 189, 194, 195, 196, 199, 206, 209
Total TEQs (7)	Dioxins: TCDD, D1, D2, D3, D4, D5, D6, D7 Furans: F1, F2, F3, F4, F5, F6, F7, F8, F9, F10 PCBs: 81, 105, 118, 126, 156, 157, 167, 169, 189
MC-Type Inducer PCBs (10)	PCBs: 66, 74, 105, 110, 118, 126, 156, 167 (70, 77, 107)
Mixed-type PCBs (10)	PCBs: 99, 128, 138, 157, 167, 170, 177 (158, 171)
PB-Type Inducer PCBs (10)	44, 49, 52, 87, 99, 101, 110, 149, 151, 153, 177, 180, 183, 187, 194, 195, 196, 199, 206 (17, 31, 82, 91, 92, 95, 97, 132, 136, 141, 174, 179, 193, 201, 205)

[a] PCB = Polychlorinated Biphenyl, TEQ = Toxic Equivalents, CYP = Cytochrome P450, TCDD = 2,3,7,8-Tetrachlorodibenzodioxin, D = Dioxin, F = Furan, MC = 3-methyl-cholanthrene, PB = phenobarbital

[b] PCB congeners in parentheses are included in the method for PCB grouping, but were not measured in any cycles from 1999 to 2004

Circulating sex hormones were measured in males age 12 and older of Subsample C. Participants from subsample C with remaining serum available in the repository were used to measure total testosterone, total estradiol, SHBG, and 3α-ADG. The free and SHBG-bound fractions of testosterone and estradiol were estimated using methods described elsewhere (41). Other laboratory analytes included serum cholesterol and triglycerides (lipids), hemoglobin A1c, serum albumin, and serum creatinine. These analytes were measured in participants age 12 and older of the full MEC sample using methods described elsewhere (40). Due to lipophilicity of PCBs, it is recommended that PCBs are adjusted for serum total lipids, which was calculated using the following formula (42):

Equation 3: $\text{Total Lipids} = (2.27 \times \text{Total Cholesterol}) + \text{Triglycerides} + 62.3 \text{ mg/dl}$

5.2.4 Statistical analysis

The analytic sample was restricted to male participants aged 20+ who did not report use of prescription hormone modifying drugs (exogenous sex hormones, thyroid hormones, 5-alpha-reductase inhibitors, growth hormones, prolactin inhibitors, antigonadotropic agents, selective estrogen receptor modulators, antiandrogens, anithyroid agents, aromatase inhibitors, and glucocorticoids) in the 30 days prior to medical examination. Exclusions to the analytic sample can be seen in Figure 1. Due to limitations of the laboratory analysis, selected PCB congeners, dioxins, and furans were missing in some participants. These data were assumed to be missing completely at random. PCB congeners that were not measured consistently across NHANES cycles were assumed to be missing at random since the only predictor of missingness was survey cycle. Missing data for PCBs, dioxins, furans, and all sex hormones were imputed through

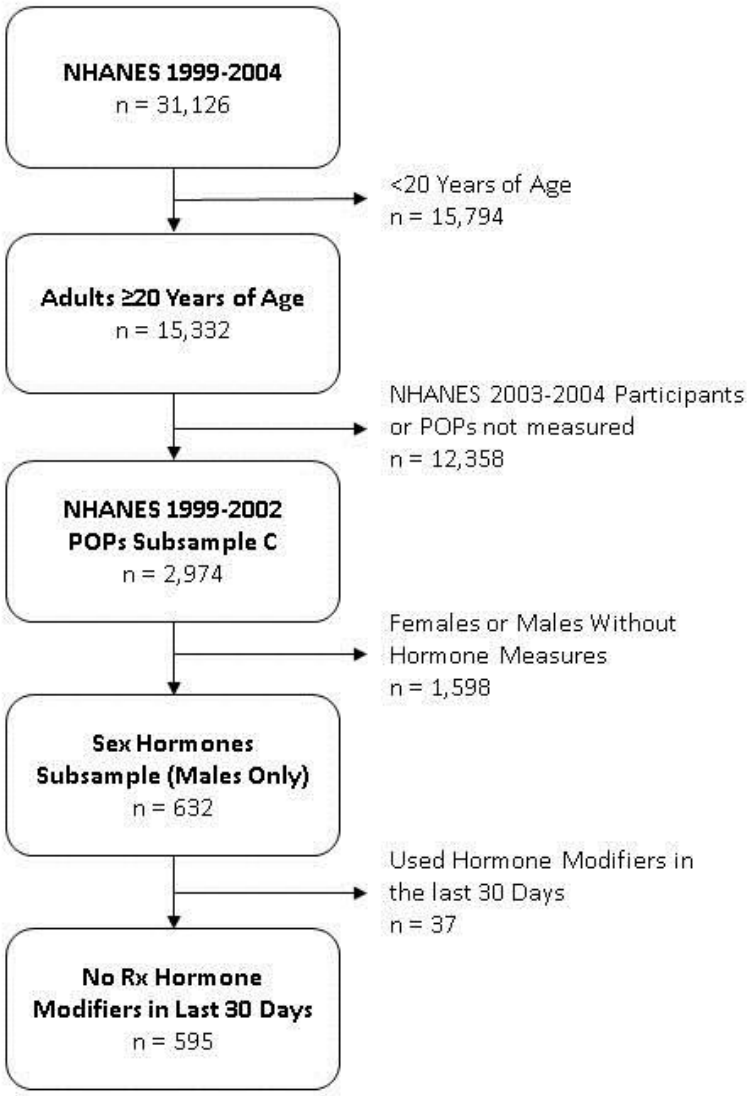

Figure 4. Analytic sample inclusions and exclusions [a]

[a] NHANES = National Health and Nutrition Examination Survey, POPs = Persistent Organic Pollutants, Rx = Prescription

predictive mean matching using multiple imputation by chained equations (MICE) package in R (43).

Missing data on demographic and health characteristics were also imputed. Data from the 2002-2003 NHANES cycle was used in addition to the 1999-2000 and 2001-2002 NHANES cycles to inform the imputations of missing data in the 1999-2000 and 2001-2002 cycles for 5 iterations of 20 imputed datasets. Missing data was imputed separately for each age group to account for potential interactions with age. Prior to imputation of missing data, there was 1 (<1%) participant missing data on education, 14 (2%) on BMI, 1 (<1%) on smoking, and 47 (8%) on alcohol consumption. All 595 participants were missing at least 1 PCB congener between the three cycles since congeners unique to 2003-2004 were imputed for 1999-2002. See Appendix B for details on imputation methodology. Descriptive statistics were calculated as n (%) for demographic and health variables while geometric means and 95% confidence intervals were calculated for serum sex hormones and PCB groupings, both overall and stratified by demographic and health characteristics. Geometric means of serum sex hormone and PCB groupings were compared across categories of demographic and health characteristics using linear regression models of each demographic/health characteristic on each hormone/PCB grouping.

Linear regression models were used to regress individual circulating sex hormone measures on each serum PCB classification. All PCB classifications and sex hormone measures were log-normally distributed and therefore were natural log transformed to meet the assumptions of linear regression. Tertiles of PCB classifications confirmed a general log-linear dose-response of PCB groupings with log-hormones. The result of such transformations is a change in the interpretation of beta coefficients to be “the percent change in Y for each 1%

increase in X" and all beta coefficients were multiplied by 50 to represent a 50% increase in PCBs. The saturated model included survey cycle, age, race/ethnicity, body mass index (BMI), education, smoking, alcohol consumption, and serum lipids. Model covariates that were non-confounders of the main association were dropped from the final model if the p-value for the beta coefficient was greater than 0.3. Despite well documented associations PCBs with diabetes, comorbid diabetes was not assessed as a confounder due to its potentially complex bidirectional mediation role between PCBs and sex hormones.

To account for estradiol and testosterone feedback regulation of SHBG, sensitivity analyses were included if there were significant associations of PCBs with either estradiol or testosterone and PCBs with SHBG where models of PCBs on SHBG were additionally adjusted for either estradiol or testosterone, respectively. Effect modification by age was assessed by inclusion of an interaction term between each PCB grouping and age group, using the youngest age group as the reference category. Effect modification by diabetes was assessed by excluding to participants without diabetes. All estimates accounted for multiply imputed datasets and NHANES multi-level survey sampling design using "mi svyset:" in combination with the "miestimate: svy:" prefix in Stata v13.1.

5.3. Results

The final analytic sample included 595 male participants which, after applying NHANES weights, consisted mainly of young (44%) and middle-aged (40%) overweight (43%) or obese (25%) non-Hispanic white males (69%). Most participants had at least some college education (55%), were non-smokers (72%), and most reported consuming alcohol in the last year (75%). Weighted and unweighted demographic characteristics of the analytic sample can be seen in Table XIV.

TABLE XIV. WEIGHTED AND UNWEIGHTED DEMOGRAPHIC AND HEALTH CHARACTERISTICS [a]

Variable	Unimputed/ Unweighted n (%)	Imputed/ Weighted %
Overall	595 (100.0)	100.00
Survey Cycle		
1999-2000	236 (39.7)	39.4
2001-2002	359 (60.3)	60.6
Age Groups		
20-39	225 (37.8)	43.5
40-59	199 (33.5)	39.9
60-85	171 (28.7)	16.6
Race/Ethnicity		
Non-Hispanic White	288 (48.4)	68.8
Non-Hispanic Black	112 (18.8)	10.7
Mexican-American	140 (23.5)	8.3
Other	55 (9.2)	12.2
Education		
Less than HS	197 (33.2)	21.4
HS Grad or Equivalent	119 (20.0)	23.3
More than HS	278 (46.8)	55.3
Missing	1	-
Body Mass Index (BMI)		
Normal/Underweight	185 (31.8)	32.0
Overweight	248 (42.7)	42.7
Obese	148 (25.5)	25.2
Missing	14	-
Smoking		
Never	247 (41.6)	43.2
Former	189 (31.8)	28.6
Current	158 (26.6)	28.2
Missing	1	-
Alcohol		
No	137 (25.0)	25.3
Yes	411 (75.0)	74.7
Missing	47	-

[a] HS = high school.

Increasing age was associated with reduced total and free testosterone, total and free estradiol, and 3α-ADG as well as elevated SHBG and SHBG-bound estradiol. Non-Hispanic whites had generally lower free and total testosterone than all other race/ethnicities while Non-Hispanic blacks had higher concentrations of all hormone measures except 3α-ADG. Those with less than a high school education had elevated total testosterone, SHBG-bound testosterone, SHBG-bound estradiol, and SHBG compared to both high school graduates and those with at least some college. Higher BMI was associated with reduced free, SHBG-bound, and total testosterone, SHBG-bound estradiol, and SHBG while higher BMI was associated with elevated free estradiol and 3α-ADG.

Current smokers, when compared to never-smokers, had significantly higher concentrations of all hormone measures except 3α-ADG, which was slightly lower. In contrast, compared to non-smokers, former smokers had lower free, SHBG-bound, and total testosterone as well as lower free and total estradiol while they had significantly higher SHBG. Higher serum lipids were associated with lower concentrations of all hormones except 3α-ADG, which was positively associated with serum lipids. Hormones were not associated with survey cycle, or alcohol consumption (Table XV).

Increasing age was associated with higher concentrations of all PCB classifications as well as TEQs. Hispanics had PCB concentrations lower than non-Hispanic whites while non-Hispanic blacks had generally higher concentrations of PCBs. Serum PCBs were elevated in former smokers compared to never smokers but were similar in current smokers compared to never smokers. PCBs were not consistently associated with BMI, though it should be noted that PCB were lipid-adjusted in all classifications. PCBs were also not associated with survey cycle, education or alcohol consumption (Table XVI).

All regression models were adjusted for age groups, BMI categories, race/ethnicity, education, survey cycle, smoking status, and serum lipids. A 50% increase in PB-type inducer PCBs and total PCBs were associated with an increase of 8.1% (95% CI: 2.2, 14.1; p=0.009) and 6.5% (95% CI: 0.3, 12.8; p=0.041) in SHBG, respectively, and these associations did not differ between age groups (Table XVII).

However, some associations were detected only in the oldest age group (XVIII). These include a 50% increase in PB-type inducer PCBs that was associated with an 18.9% (95% CI: 0.4, 37.5; p=0.046) increase in total testosterone, a 20.7% (95% CI: 1.7, 39.7; p=0.034) increase in SHBG-bound testosterone, and, and a 16.2% (95% CI: 1.0, 31.4; p=0.038) increase in the testosterone to estradiol ratio. PCBs were not associated with free testosterone, total estradiol, free estradiol, or SHBG-bound estradiol in any age group.

Since elevated testosterone is known to inhibit production of SHBG, a sensitivity analysis was performed where SHBG was regressed on PCB groupings, additionally adjusted for total testosterone. After additionally adjusting for total testosterone, a 50% increase in PB-type inducer PCBs was associated with a 9.3% (95% CI: 0.6, 18.0; p=0.037) increase in SHBG in the youngest age group, a 6.5% (95% CI: -1.0, 14.1; p=0.085) increase in SHBG in the middle age group, and a 1.4% (95% CI: -8.1, 10.9; p=0.762) increase in the oldest age group.

A 50% increase in 4 of the 5 PCB groupings were associated with a decrease in 3α-ADG overall (XIV). After stratifying by age, the associations were strongest in males age 20-39 years and near null among males age 60-85 years (Table XVIII). Among males age 20-39 years, the magnitude of estimates ranged from the largest decrease in 3α-ADG of 18.2% (95% CI: -28.4, -7.9; p=0.001) with a 50% increase in PB-type inducer PCBs to a 10.4% (-20.3, -0.5; p=0.040) decrease in 3α-ADG with a 50% increase in MC type inducer PCBs. Similar associations were

TABLE XV. BIVARIATE ASSOCIATIONS OF DEMOGRAPHIC AND HEALTH CHARACTERISTICS WITH CIRCULATING SEX HORMONES [a]

	Total T (ng/dL)		Total E2 (pg/mL)		3α-ADG (ng/mL)		SHBG (nmol/L)	
Variable	Geomean (95% CI)	p-value [b]	Geomean (95% CI)	p-value [b]	Geomean (95% CI)	p-value [b]	Geomean (95% CI)	p-value [b]
Overall	460.8 (439.5-483.1)	-	26.3 (24.8-27.9)	-	7.15 (6.78-7.54)	-	33.1 (31.5-34.8)	-
Survey Cycle								
1999-2000	486.8 (458.7-516.7)	Ref	26.4 (23.7-29.4)	Ref	6.77 (6.19-7.42)	Ref	33.7 (31.3-36.2)	Ref
2001-2002	444.6 (415.3-475.9)	0.049	26.2 (24.4-28.1)	0.926	7.41 (6.95-7.9.0)	0.111	32.8 (30.6-35.0)	0.566
Age Groups								
20-39	542.3 (509.0-577.7)	Ref	28.6 (26.5-30.9)	Ref	7.35 (6.89-7.85)	Ref	28.3 (26.1-30.7)	Ref
40-59	436.5 (407.4-467.7)	<0.001	26.1 (23.9-28.5)	0.077	7.49 (6.8.0-8.26)	0.753	32.7 (30.5-35.0)	0.011
60-85	342.6 (308.5-380.5)	<0.001	21.5 (19.9-23.2)	<0.001	5.93 (5.32-6.62)	0.001	51.6 (47.0-56.7)	<0.001
Race/Ethnicity								
Non-Hispanic White	442.4 (415.4-471.2)	Ref	25.2 (23.3-27.3)	Ref	6.95 (6.43-7.52)	Ref	33.0 (30.9-35.2)	Ref
Mexican-American	477.6 (429.0-531.5)	0.197	24.9 (22.2-27.8)	0.803	7.46 (6.77-8.23)	0.283	29.6 (26.6-32.9)	0.105
Non-Hispanic Black	541.8 (487.7-601.9)	0.003	34.4 (31.2-38.0)	<0.001	7.46 (6.77-8.23)	0.26	38.0 (33.7-42.8)	0.056
Other	490.2 (439.2-547.2)	0.109	27.3 (23.9-31.1)	0.304	7.83 (6.49-9.45)	0.279	32.2 (27.9-37.1)	0.728
Education								
Less than HS	509.7 (469.3-553.6)	Ref	27.1 (24.8-29.7)	Ref	7.45 (6.89-8.05)	Ref	38.5 (35.7-41.5)	Ref
HS Grad or Equivalent	431.1 (386.6-480.7)	0.031	27.4 (23.7-31.6)	0.912	7.21 (6.30-8.26)	0.7	30.6 (27.0-34.6)	0.005
More than HS	455.7 (421.9-492.2)	0.041	25.5 (23.6-27.6)	0.184	7.01 (6.55-7.5.0)	0.235	32.3 (30.4-34.3)	0.001
Body Mass Index (BMI)		.		.		.		.
Normal/Underweight	567.9 (527.2-611.8)	Ref	26.8 (24.4-29.4)	Ref	6.74 (6.23-7.28)	Ref	42.1 (38.8-45.7)	Ref
Overweight	445.7 (417.9-475.5)	<0.001	24.4 (23.0-26.0)	0.035	6.83 (6.29-7.42)	0.82	31.0 (29.3-32.8)	<0.001
Obese	373.8 (336.2-415.6)	<0.001	29.0 (25.7-32.8)	0.273	8.34 (7.30-9.52)	0.011	27.3 (23.9-31.1)	<0.001
Smoking								
Never	475.2 (446.1-506.1)	Ref	27.0 (25.6-28.4)	Ref	7.65 (7.01-8.34)	Ref	30.7 (28.4-33.1)	Ref
Former	365.6 (336.0-397.9)	<0.001	20.0 (18.2-22.1)	<0.001	6.66 (6.04-7.34)	0.057	35.3 (31.8-39.1)	0.036
Current	555.6 (522.2-591.1)	0.003	33.3 (30.2-36.7)	<0.001	6.94 (6.28-7.66)	0.09	34.9 (32.1-38.0)	0.038
Alcohol								
No	437.1 (389.3-490.7)	Ref	25.9 (23.3-28.9)	Ref	7.36 (6.70-8.08)	Ref	34.4 (30.8-38.3)	Ref
Yes	469.1 (445.5-494.0)	0.267	26.4 (24.3-28.7)	0.809	7.08 (6.56-7.64)	0.574	32.7 (30.7-34.8)	0.46
Lipids								
Tertile 1	529.5 (493.2-568.4)	Ref	31.0 (28.7-33.6)	Ref	6.51 (5.95-7.12)	Ref	36.1 (33.3-39.2)	Ref
Tertile 2	432.3 (396.9-470.8)	<0.001	24.1 (21.8-26.6)	<0.001	7.34 (6.71-8.04)	0.061	32.9 (29.8-36.4)	0.145
Tertile 3	429.4 (396.1-465.5)	<0.001	24.4 (22.1-26.9)	0.001	7.60 (6.88-8.40)	0.028	30.8 (28.5-33.3)	0.004

[a] T = Testosterone; E2 = Estradiol; 3α-ADG = 3α-androstanediol glucuronide; Geomean = Geometric Mean, CI = Confidence Interval, Ref = Reference, 3α-ADG = 3-Alpha Androstanediol Glucuronide, SHBG = Steroid Hormone Binding Globulin, HS = High School

[b] p-value for t-test of difference in log-mean from reference category

TABLE XV (CONTINUED). BIVARIATE ASSOCIATIONS OF DEMOGRAPHIC AND HEALTH CHARACTERISTICS WITH CIRCULATING SEX HORMONES [a]

	SHBG-bound T (ng/dL)		Free T (ng/dL)		SHBG-bound E2 (pg/mL)		Free E2 (pg/mL)	
Variable	Geomean (95% CI)	p-value [b]	Geomean (95% CI)	p-value [b]	Geomean (95% CI)	p-value [b]	Geomean (95% CI)	p-value [b]
Overall	224.6 (211.4-238.6)	-	9.12 (8.66-9.6)	-	6.3 (5.96-6.66)	-	0.478 (0.446-0.513)	-
Survey Cycle								
1999-2000	235.4 (218.3-253.8)	Ref	9.44 (8.85-10.06)	Ref	6.25 (5.74-6.8)	Ref	0.468 (0.415-0.527)	Ref
2001-2002	217.9 (199.8-237.5)	0.178	8.91 (8.26-9.62)	0.248	6.33 (5.88-6.82)	0.805	0.485 (0.446-0.527)	0.612
Age Groups								
20-39	235.4 (213.9-259.2)	Ref	11.86 (11.28-12.47)	Ref	5.71 (5.2-6.26)	Ref	0.544 (0.501-0.59)	Ref
40-59	216.6 (198.6-236.3)	0.186	8.75 (8.17-9.38)	<0.001	6.38 (5.8-7.01)	0.087	0.482 (0.439-0.53)	0.023
60-85	216.6 (190.9-245.7)	0.253	5.05 (4.64-5.5)	<0.001	7.92 (7.17-8.74)	<0.001	0.335 (0.304-0.369)	<0.001
Race/Ethnicity								
Non-Hispanic White	215.8 (200.4-232.3)	Ref	8.7 (8.05-9.39)	Ref	6.07 (5.69-6.49)	Ref	0.456 (0.416-0.499)	Ref
Mexican-American	216.7 (188.5-249.1)	0.957	10.05 (9.04-11.18)	0.032	5.36 (4.62-6.21)	0.14	0.461 (0.408-0.521)	0.844
Non-Hispanic Black	281.7 (247.6-320.6)	0.001	10.24 (9.19-11.4)	0.031	8.91 (8.04-9.86)	<0.001	0.623 (0.55-0.706)	0.001
Other	236.5 (199.8-279.8)	0.293	10.05 (9.29-10.86)	0.017	6.36 (5.3-7.64)	0.621	0.509 (0.444-0.585)	0.17
Education								
Less than HS	266.2 (241.6-293.2)	Ref	9.33 (8.53-10.22)	Ref	7.24 (6.58-7.98)	Ref	0.473 (0.426-0.526)	Ref
HS Grad or Equivalent	201.4 (180.3-225.1)	0.001	8.84 (7.63-10.24)	0.563	6.13 (5.49-6.85)	0.058	0.507 (0.43-0.599)	0.512
More than HS	220.2 (201.5-240.6)	0.005	9.15 (8.43-9.93)	0.731	6.03 (5.52-6.59)	0.002	0.468 (0.431-0.508)	0.826
Body Mass Index (BMI)								
Normal/Underweight	303.1 (276.7-332)	Ref	9.99 (9.11-10.95)	Ref	7.37 (6.72-8.07)	Ref	0.454 (0.406-0.508)	Ref
Overweight	212.5 (196.4-230)	<0.001	9.15 (8.55-9.8)	0.13	5.68 (5.29-6.1)	<0.001	0.453 (0.425-0.483)	0.963
Obese	168.7 (145.4-195.6)	<0.001	8.07 (7.29-8.93)	0.004	6.16 (5.41-7)	0.023	0.559 (0.486-0.642)	0.016
Smoking								
Never	221.5 (202.7-242)	Ref	9.83 (9.3-10.4)	Ref	6.02 (5.57-6.51)	Ref	0.5 (0.472-0.531)	Ref
Former	189.7 (171.1-210.2)	0.016	6.8 (6.17-7.49)	<0.001	5.39 (4.89-5.94)	0.095	0.351 (0.313-0.395)	<0.001
Current	272.3 (249.9-296.8)	0.004	10.92 (10.15-11.75)	0.041	7.9 (7.12-8.76)	<0.001	0.609 (0.545-0.681)	<0.001
Alcohol								
No	218.8 (191.3-250.1)	Ref	8.41 (7.39-9.57)	Ref	6.5 (5.69-7.43)	Ref	0.465 (0.413-0.524)	Ref
Yes	226.6 (210.7-243.7)	0.652	9.37 (8.87-9.9)	0.125	6.23 (5.74-6.76)	0.634	0.483 (0.438-0.532)	0.663
Lipids								
Tertile 1	265.5 (241.5-291.8)	Ref	10.17 (9.43-10.96)	Ref	7.66 (7.07-8.3)	Ref	0.558 (0.508-0.612)	Ref
Tertile 2	210.4 (188.7-234.6)	0.001	8.47 (7.67-9.34)	0.005	5.83 (5.13-6.62)	0.001	0.434 (0.389-0.484)	0.001
Tertile 3	204.4 (186.8-223.7)	<0.001	8.8 (8.04-9.63)	0.021	5.65 (5.28-6.04)	<0.001	0.452 (0.402-0.508)	0.012

[a] T = Testosterone; E2 = Estradiol; 3α-ADG = 3α-androstanediol glucuronide; Geomean = Geometric Mean, CI = Confidence Interval, Ref = Reference, 3α-ADG = 3-Alpha Androstanediol Glucuronide, SHBG = Steroid Hormone Binding Globulin, HS = High School

[b] p-value for t-test of difference in log-mean from reference category

TABLE XVI. BIVARIATE ASSOCIATIONS OF DEMOGRAPHIC AND HEALTH CHARACTERISTICS WITH PCB GROUPINGS AND TEQS [a]

	Total PCBs		Total TEQ	
Variable	*Geomean (95% CI)*	*p-value* [b]	*Geomean (95% CI)*	*p-value* [b]
Overall	292.5 (280.6-305)	-	26.8 (25.5-28.2)	-
Survey Cycle				
1999-2000	291.6 (276.7-307.2)	Ref	26.3 (24.9-27.8)	Ref
2001-2002	293.2 (276-311.5)	0.887	27.1 (25.2-29.2)	0.479
Age Groups				
20-39	232.1 (218.9-246.1)	Ref	22.3 (20.7-24)	Ref
40-59	309.9 (290.4-330.7)	<0.001	27.3 (25.5-29.3)	<0.001
60-85	466.7 (437.1-498.4)	<0.001	41.4 (38.2-44.8)	<0.001
Race/Ethnicity				
Non-Hispanic White	301.3 (287.6-315.7)	Ref	27.4 (25.9-29.1)	Ref
Mexican-American	222.2 (207.2-238.3)	<0.001	21.9 (20.5-23.4)	<0.001
Non-Hispanic Black	345.9 (305.3-391.8)	0.042	29.8 (26.3-33.7)	0.16
Other	257.8 (224-296.8)	0.048	24.6 (22.1-27.3)	0.074
Education				
Less than HS	300.4 (273-330.5)	Ref	27.6 (25.2-30.4)	Ref
HS Grad or Equivalent	286 (261.7-312.5)	0.458	26.4 (24-29)	0.424
More than HS	292.3 (277.5-308)	0.63	26.7 (25.1-28.3)	0.492
Body Mass Index (BMI)		.		.
Normal/Underweight	311 (294-329)	Ref	26.8 (25.2-28.6)	Ref
Overweight	282.2 (264.4-301.3)	0.027	26 (24.3-27.8)	0.446
Obese	287.7 (258-320.8)	0.176	28.2 (24.9-31.8)	0.41
Smoking				
Never	277.5 (259.6-296.7)	Ref	26 (24.3-27.9)	Ref
Former	328 (304.2-353.7)	<0.001	30.3 (28.2-32.6)	0.001
Current	282.5 (263.5-302.8)	0.724	24.7 (22.8-26.8)	0.188
Alcohol				
No	300.8 (272.1-332.5)	Ref	27.3 (24.7-30.1)	Ref
Yes	289.8 (275.9-304.4)	0.523	26.6 (25.3-28)	0.626
Lipids				
Tertile 1	330.2 (305.8-356.4)	Ref	29.7 (27.1-32.5)	Ref
Tertile 2	293.6 (275.9-312.5)	0.015	27.1 (25.2-29)	0.08
Tertile 3	262.1 (243.3-282.2)	<0.001	24.3 (22.5-26.2)	0.001

[a] PCB = polychlorinated biphenyl; MC = 3-methylcholanthrene; PB = phenobarbital; Geomean = geometric mean; CI = confidence interval; Ref. = reference category; BMI = body mass index; HS = high school

[b] p-value for t-test of difference in log-mean from reference category

TABLE XVI (CONTINUED). BIVARIATE ASSOCIATIONS OF DEMOGRAPHIC AND HEALTH CHARACTERISTICS WITH PCB GROUPINGS AND TEQS [a]

	MC-Type PCBs		Mixed-Type PCBs		PB-Type PCBs	
Variable	*Geomean (95% CI)*	*p-value* [b]	*Geomean (95% CI)*	*p-value* [b]	*Geomean (95% CI)*	*p-value* [b]
Overall	39.8 (38-41.6)	-	57.8 (54.9-60.8)	-	171.6 (164-179.6)	-
Survey Cycle						
1999-2000	41.5 (39.2-43.9)	Ref	58.1 (54.8-61.6)	Ref	172.9 (162.9-183.6)	Ref
2001-2002	38.7 (36.3-41.2)	0.101	57.5 (53.3-62)	0.82	170.8 (160.2-182.1)	0.775
Age Groups						
20-39	33.7 (31.3-36.2)	Ref	42.8 (40.1-45.8)	Ref	134.6 (126.7-143)	Ref
40-59	39.4 (36.6-42.5)	0.007	64.6 (60.2-69.2)	<0.001	183.5 (170.6-197.4)	<0.001
60-85	62.6 (57.3-68.4)	<0.001	96.7 (88.8-105.3)	<0.001	275.9 (258.3-294.8)	<0.001
Race/Ethnicity						
Non-Hispanic White	41.1 (39.1-43.3)	Ref	59.9 (56.6-63.4)	Ref	176.9 (168.2-186.1)	Ref
Non-Hispanic Black	32.2 (30.1-34.4)	<0.001	40.5 (37.3-44)	<0.001	127.8 (118.3-138.1)	<0.001
Mexican-American	45 (39-51.9)	0.207	68.5 (58.4-80.2)	0.109	205.6 (182.7-231.4)	0.025
Other	33.9 (29.9-38.5)	0.013	51.6 (43.6-60.9)	0.111	150.9 (129.5-175.9)	0.055
Education						
Less than HS	39.8 (36.1-43.9)	Ref	59.4 (52.6-67)	Ref	177.4 (160.6-195.9)	Ref
HS Grad or Equivalent	38.8 (35.3-42.5)	0.681	57 (51.7-63)	0.612	167.9 (152.8-184.4)	0.413
More than HS	40.1 (37.8-42.6)	0.896	57.4 (53.8-61.3)	0.641	171 (162-180.6)	0.523
Body Mass Index (BMI)		.		.		.
Normal/Underweight	40.3 (37.8-42.9)	Ref	59.3 (55.2-63.7)	Ref	185.5 (174.8-196.8)	Ref
Overweight	37.9 (35.4-40.5)	0.113	55.9 (51.7-60.5)	0.271	165.9 (155.2-177.4)	0.015
Obese	42.4 (37.9-47.5)	0.414	59 (51.7-67.3)	0.945	164.6 (146.4-185.1)	0.055
Smoking						
Never	39.8 (36.9-42.8)	Ref	53.7 (49.1-58.6)	Ref	161 (150.3-172.5)	Ref
Former	43.8 (40.2-47.8)	0.042	66.7 (61.5-72.3)	<0.001	193.5 (178.5-209.8)	<0.001
Current	36 (33.5-38.6)	0.062	55.9 (51.5-60.6)	0.479	167.6 (155.9-180.1)	0.436
Alcohol						
No	40.8 (37-45.1)	Ref	59.6 (52.8-67.3)	Ref	175.8 (157.9-195.7)	Ref
Yes	39.4 (37.5-41.3)	0.503	57.1 (54-60.5)	0.528	170.2 (161.3-179.7)	0.61
Lipids						
Tertile 1	45.9 (42.2-50.1)	Ref	62.2 (56.4-68.5)	Ref	193.3 (178.7-209.1)	Ref
Tertile 2	39.8 (37.5-42.1)	0.004	57.6 (53.1-62.4)	0.168	171.9 (160.8-183.9)	0.021
Tertile 3	35 (32.5-37.6)	<0.001	54.2 (50-58.9)	0.023	154.2 (142.3-167.2)	<0.001

[a] PCB = polychlorinated biphenyl; MC = 3-methylcholanthrene; PB = phenobarbital; Geomean = geometric mean; CI = confidence interval; Ref. = reference category; BMI = body mass index; HS = high school

[b] p-value for t-test of difference in log-mean from reference category

observed among males age 40-59 years, but at smaller magnitudes compared to participants age 20-40 years and statistically significant in only 6 of the 7 PCB groupings ($p<0.05$). Among participants age 60-80 years and for all PCB groupings, we observed no significant change in 3α-ADG. After standardizing 3α-ADG concentrations to total testosterone concentrations using the 3α-ADG to total testosterone ratio, these associations become more consistent across age groups. Results did not change after excluding to participants without diabetes (data not shown).

5.4. Discussion

This study demonstrated associations of PCBs with sex hormones in a representative sample of the non-institutionalized US male population. However, these associations differ in direction and magnitude depending on the PCB grouping method, the target hormone, and age group. To the best of our knowledge, the association between PCB exposures and concentrations of male sex hormones has not been investigated using this data from these NHANES cycles.

Much of the past toxicological literature has focused estrogen receptor binding (i.e. estrogenicity) as the most plausible mechanism of action for PCB affecting sex hormones (8,9). However, more recent research has identified PCB congeners which may directly, or through their metabolites, influence synthesis of cytochrome P420 enzymes, which play major roles in sex hormone metabolism (10,44). Thus, it is important to highlight the strong overlap between congeners included in estrogenicity-based PCB groupings and CYP-based PCB groupings to make comparisons between this study and studies conducted in the past. Specifically, the estrogenic and the anti-estrogenic PCB groups developed by Wolff et al. contains many of the same congeners as the PB-type inducer and MC type inducer PCB groups developed by Warner et al., respectively.

TABLE XVII. MODEL-ADJUSTED PERCENT CHANGE IN HORMONES PER 50% INCREASE IN PCB GROUPING [a]

Hormone	**Overall**	
PCB Grouping	% Change (95% CI) [b]	p-value [c]
SHBG		
Total PCBs	6.5 (0.3, 12.8)	0.041
Total TEQs	3.1 (-3.4, 9.5)	0.336
MC Type Inducers	2.3 (-3.3, 8.0)	0.407
Mixed Type Inducers	3.3 (-1.9, 8.6)	0.200
PB Type Inducers	8.1 (2.2, 14.1)	0.009
SHBG (adj. Total T) [d]		
Total PCBs	5.4 (-0.5, 11.3)	0.071
Total TEQs	3.4 (-2.7, 9.5)	0.266
MC Type Inducers	2.6 (-2.8, 8.1)	0.327
Mixed Type Inducers	3.0 (-2.3, 8.3)	0.254
PB Type Inducers	6.5 (0.8, 12.2)	0.026
Total T		
Total PCBs	3.6 (-1.7, 8.9)	0.175
Total TEQs	-1.0 (-5.5, 3.6)	0.664
MC Type Inducers	-1.0 (-5.4, 3.4)	0.641
Mixed Type Inducers	1.1 (-2.8, 4.9)	0.568
PB Type Inducers	5.2 (-0.3, 10.6)	0.063
SHBG-bound T		
Total PCBs	7.0 (0.2, 13.9)	0.044
Total TEQs	0.7 (-5.4, 6.7)	0.822
MC Type Inducers	0.2 (-5.4, 5.9)	0.929
Mixed Type Inducers	3.0 (-1.6, 7.7)	0.195
PB Type Inducers	9.5 (2.5, 16.4)	0.009
Free T		
Total PCBs	-0.1 (-6.2, 6.0)	0.972
Total TEQs	-3.0 (-8.5, 2.5)	0.277
MC Type Inducers	-2.7 (-8.2, 2.8)	0.328
Mixed Type Inducers	-0.9 (-6.1, 4.3)	0.735
PB Type Inducers	0.8 (-5.3, 6.8)	0.800
3*α*-ADG		
Total PCBs	-10.2 (-16.4, -4.1)	0.002
Total TEQs	-10.4 (-15.8, -4.9)	0.001
MC Type Inducers	-4.2 (-10.0, 1.6)	0.147
Mixed Type Inducers	-7.6 (-12.1, -3.0)	0.002
PB Type Inducers	-11.2 (-17.4, -4.9)	0.001
3*α*-ADG to Total T Ratio		
Total PCBs	-13.8 (-20.7, -6.9)	<0.001
Total TEQs	-9.4 (-16.5, -2.4)	0.011
MC Type Inducers	-3.2 (-9.3, 3.0)	0.295
Mixed Type Inducers	-8.6 (-14, -3.3)	0.003
PB Type Inducers	-16.4 (-23.4, -9.3)	<0.001

[a] CI = confidence interval, PCB = polychlorinated biphenyl, TEQ = toxic equivalents, MC = 3-methylcholanthrene, PB = phenobarbital, SHBG = steroid hormone binding globulin, T = testosterone, E2 = estradiol, 3α-ADG = 3-alpha-androstanediol glucuronide

[b] Estimates represent the percent change in hormone measure per 50% increase in PCBs, adjusted for survey cycle, age, race/ethnicity, BMI, education, smoking, and serum lipids

[c] p-value for % change = 0

[d] Additionally adjusted for Total T

TABLE XVII (CONTINUED). MODEL-ADJUSTED PERCENT CHANGE IN HORMONES PER 50% INCREASE IN PCB GROUPING [a]

Hormone	**Overall**	
PCB Grouping	% Change (95% CI) [b]	p-value [c]
Total E2		
Total PCBs	-1.5 (-9.4, 6.4)	0.404
Total TEQs	1.3 (-5.1, 7.7)	0.956
MC Type Inducers	0.9 (-6.5, 8.2)	0.998
Mixed Type Inducers	-0.7 (-6.8, 5.3)	0.585
PB Type Inducers	-2.5 (-10.4, 5.4)	0.242
SHBG-bound E2		
Total PCBs	3.6 (-4.3, 11.4)	0.362
Total TEQs	3.6 (-3.5, 10.7)	0.306
MC Type Inducers	2.4 (-5.5, 10.4)	0.535
Mixed Type Inducers	2.0 (-4.0, 8.0)	0.500
PB Type Inducers	3.8 (-4.0, 11.7)	0.325
Free E2		
Total PCBs	-3.6 (-12.5, 5.2)	0.410
Total TEQs	0.2 (-7.0, 7.4)	0.966
MC Type Inducers	0.0 (-8.0, 8.0)	0.927
Mixed Type Inducers	-1.9 (-8.8, 5.1)	0.579
PB Type Inducers	-5.2 (-14.0, 3.7)	0.254
Total T to Total E2 Ratio		
Total PCBs	5.0 (-1.9, 12.0)	0.147
Total TEQs	-2.3 (-8.3, 3.7)	0.441
MC Type Inducers	-1.9 (-7.6, 3.9)	0.510
Mixed Type Inducers	1.8 (-3.7, 7.4)	0.503
PB Type Inducers	7.7 (0.7, 14.6)	0.031

[a] CI = confidence interval, PCB = polychlorinated biphenyl, TEQ = toxic equivalents, MC = 3-methylcholanthrene, PB = phenobarbital, SHBG = steroid hormone binding globulin, T = testosterone, E2 = estradiol, 3α-ADG = 3-alpha-androstanediol glucuronide

[b] Estimates represent the percent change in hormone measure per 50% increase in PCBs, adjusted for survey cycle, age, race/ethnicity, BMI, education, smoking, and serum lipids

[c] p-value for % change = 0

[d] Additionally adjusted for Total T

TABLE XVIII. MODEL-ADJUSTED PERCENT CHANGE IN HORMONES PER 50% INCREASE IN PCB GROUPING BY AGE GROUP [a]

Hormone PCB Grouping	**20-39 years** % Change (95% CI) [bc]	**40-59 years** % Change (95% CI) [bc]	**60-85 years** % Change (95% CI) [bc]	**Strength of Effect Modification by Age** [d]
SHBG				
Total PCBs	7.4 (-3.8, 18.6)	4.9 (-2.8, 12.7)	8.8 (-0.8, 18.4)*	-
Total TEQs	3.3 (-6.8, 13.4)	1.1 (-8.7, 11)	5.8 (-3.7, 15.4)	-
MC Type Inducers	-1.3 (-10.6, 8.0)	1.2 (-6.5, 8.9)	8.4 (-1.0, 17.8)*	-
Mixed Type Inducers	5.0 (-3.5, 13.5)	0.5 (-5.9, 6.9)	7.2 (-0.7, 15.1)*	-
PB Type Inducers	10.4 (-0.3, 21.1)*	6.8 (-0.8, 14.4)*	7.5 (-0.9, 15.9)*	-
SHBG (adj. Total T) [e]				
Total PCBs	6.7 (-2.2, 15.7)	5.1 (-2.3, 12.5)	3.9 (-7.1, 15.0)	-
Total TEQs	3.0 (-5.2, 11.2)	1.7 (-6.8, 10.1)	6.6 (-4.0, 17.2)	-
MC Type Inducers	-0.6 (-8.6, 7.4)	2.2 (-4.8, 9.2)	7.2 (-2.9, 17.3)	-
Mixed Type Inducers	5.0 (-2.1, 12.2)	1.2 (-4.9, 7.3)	3.7 (-6.5, 13.9)	-
PB Type Inducers	9.3 (0.6, 18.0) †	6.5 (-1.0, 14.1)*	1.4 (-8.1, 10.9)	Weak
Total Testosterone				
Total PCBs	2.1 (-7.5, 11.7)	-0.4 (-8.2, 7.4)	15.3 (-2.4, 33.0) *	-
Total TEQs	0.7 (-7.7, 9.1)	-1.7 (-10.7, 7.3)	-2.4 (-15.5, 10.7)	-
MC Type Inducers	-2.2 (-9.1, 4.7)	-3.0 (-10.8, 4.8)	3.7 (-8.6, 16.1)	-
Mixed Type Inducers	-0.2 (-6.9, 6.5)	-2.2 (-8.8, 4.5)	10.8 (-4.4, 25.9)	-
PB Type Inducers	3.3 (-6.4, 13.0)	0.8 (-6.4, 8.1)	18.9 (0.4, 37.5) †	Moderate
SHBG-bound T				
Total PCBs	7.1 (-7.1, 21.4)	2.4 (-6.9, 11.6)	17.8 (0.0, 35.5) †	-
Total TEQs	2.9 (-9.5, 15.3)	-1.0 (-12.7, 10.6)	-0.1 (-12.9, 12.7)	-
MC Type Inducers	-2.4 (-13.0, 8.2)	-1.9 (-11.6, 7.9)	6.9 (-5.6, 19.3)	-
Mixed Type Inducers	3.4 (-6.6, 13.4)	-1.5 (-9.2, 6.1)	13.0 (-1.4, 27.4) *	-
PB Type Inducers	10.1 (-3.8, 23.9)	4.4 (-4.1, 12.9)	20.7 (1.7, 39.7) †	-
Total T to Total E2 Ratio				
Total PCBs	2.5 (-11.8, 16.8)	4.6 (-6.1, 15.3)	10.1 (-5.7, 26.0)	-
Total TEQs	-1.6 (-15.7, 12.6)	3.1 (-6.4, 12.6)	-11.9 (-27.5, 3.6)	-
MC Type Inducers	-5.1 (-16.5, 6.2)	0.9 (-10.3, 12)	-2.7 (-16.7, 11.3)	-
Mixed Type Inducers	1.5 (-10.2, 13.2)	0.8 (-7.9, 9.6)	4.7 (-9.4, 18.8)	-
PB Type Inducers	4.8 (-8.7, 18.4)	6.2 (-4.1, 16.4)	16.2 (1.0, 31.4) †	-
***3α*-ADG**				
Total PCBs	-16.0 (-26.0, -6.0) ‡	-11.9 (-20.3, -3.5) ‡	3.0 (-13.0, 18.9)	Moderate
Total TEQs	-15.4 (-27.9, -3.0) †	-11.4 (-21.6, -1.2) †	-1.0 (-12.4, 10.4)	-
MC Type Inducers	-9.1 (-18.6, 0.4) †	-6.2 (-15.1, 2.6)	5.0 (-7.4, 17.4)	Moderate
Mixed Type Inducers	-12.2 (-18.4, -5.9) ‡	-8.7 (-15.5, -1.8) †	3.0 (-9.2, 15.3)	Strong
PB Type Inducers	-16.7 (-26.6, -6.8) ‡	-12.4 (-20.8, -4.0) ‡	1.2 (-15.4, 17.7)	Moderate
***3α*-ADG to Total T Ratio**				
Total PCBs	-18.1 (-32.0, -4.2) †	-11.5 (-20.3, -2.8) †	-12.3 (-29.5, 4.8)	-
Total TEQs	-16.2 (-31.5, -0.9) †	-9.7 (-20.0, 0.6) *	1.4 (-12.6, 15.4)	-
MC Type Inducers	-6.9 (-20.0, 6.1)	-3.2 (-12.9, 6.5)	1.2 (-12.1, 14.6)	-
Mixed Type Inducers	-12.0 (-20.9, -3.1) ‡	-6.5 (-13.7, 0.8) *	-7.8 (-22.8, 7.3)	-
PB Type Inducers	-20.0 (-33.2, -6.8) ‡	-13.2 (-21.7, -4.8) ‡	-17.7 (-34.9, -0.6) †	-

[a] CI = confidence interval, PCB = polychlorinated biphenyl, TEQ = toxic equivalents, MC = 3-methylcholanthrene, PB = phenobarbital, SHBG = steroid hormone binding globulin, T = testosterone, E2 = estradiol, 3α-ADG = 3-alpha-androstanediol glucuronide

[b] Estimates represent the percent change in hormone measure per 50% increase in PCBs, adjusted for survey cycle, age, race/ethnicity, BMI, education, smoking, and serum lipids

[c] * p-value for % change = 0 is less than 0.10; † p-value for % change = 0 is less than 0.05; ‡ p-value for % change = 0 is less than 0.01

[d] At least 1 p-value for pairwise comparison between age groups of adjusted % change estimates is <0.10 (Moderate), <0.05 (Strong), or <0.01 (Very Strong)

[e] Additionally adjusted for Total T

5.4.1 SHBG and Testosterone/Estradiol Bioavailability

Most testosterone and estradiol in the blood is bound to SHBG and albumin with only small portions circulating freely. SHBG-bound portions of testosterone and estradiol are not available for activation of hormone receptors while the free portions are entirely available. Free portions of testosterone and estradiol are maintained within a narrow hormetic window through a feedback loop where changes in free testosterone regulate synthesis of SHBG to increase or decrease availability of testosterone and estradiol.

Both elevated PB-type inducer PCBs and total PCBs were associated with a significantly higher concentration of SHBG across all age groups. In contrast, both PB-type inducer PCBs and total PCBs (to lesser extent) were associated with significantly higher concentrations of total testosterone and SHBG-bound testosterone in only the oldest age group. Since testosterone is known to inhibit production of SHBG through a feedback loop, a sensitivity analysis was performed to remove the potential for mediation by testosterone in the association of PCBs with SHBG. The results of this analysis did not change the results in the youngest two age groups but was reduced to null in the oldest age group.

These findings suggest that PCBs may affect the binding of steroid hormones or, alternatively, the synthesis of SHBG through changes in the estrogen-androgen balance (addressed later in this discussion). Removal of any mediating effects of testosterone in the proposed causal pathway of PCBs on SHBG suggests that elevated testosterone had a strong effect on the PCB-SHBG association in the oldest age group, where there was also a strong association of PCBs with total testosterone. However, the PCB-SHBG association was still observed in the youngest two age groups, where there was no association of PCBs with

testosterone. There were no observed associations of any PCB grouping with free testosterone, total estradiol, free estradiol, or SHBG-bound estradiol.

The literature around PCBs, testosterone, and SHBG provides inconsistent results and high variability with respect to study populations, biomarker measurement, and statistical methodology. In a study of young Swedish males (age 18-21), after adjusting for BMI, duration of abstinence, and smoking, the authors found no association between PCB-153 and total testosterone but the authors did find a significant increase in SHBG with increasing PCB-153 (31). In a pooled study of 749 males from Greenland (n=258), Warsaw (m=113), Sweden (n=184), and Kharkiv (n=194), there were no association of PCB-153 with free testosterone overall or in any of the 4 individual studies, but a significant positive association of PCB-153 with SHBG was observed in the Kharkiv study [β=3.60 (1.74, 5.46)] (45). Finally, in a study of 63 male workers at an electrical capacitor plant in LaSalle, IL (age 35+ yrs.), Persky et al. (33) found marginally significant ($0.5 \leq p < 0.10$) positive correlation between estrogenic PCBs and total testosterone (r=0.03) and a significant positive correlation between estrogenic PCBs and DHEAS (r=0.13). These few examples demonstrate results that coincide with the results of our study, but most of the literature demonstrates null or inverse associations of PCBs with SHBG, testosterone, and testosterone binding.

In a study of 179 adult (mean age = 50 yrs.) males of the Great Lakes Sport-Caught Fish Consumer cohort, serum total PCBs were significantly inversely associated with total testosterone (r=-0.10) and percent SHBG-bound testosterone (r=-0.23), but not SHBG or percent free testosterone, after adjusting for age, BMI, medications, years eating GL fish, and total GL fish meals (5). However, a follow-up study in a subgroup of the same cohort found no association of total PCBs or total TEQs with total testosterone (r=0.02; p=0.87) or SHBG (-0.09;

p=0.63), but did find a similar inverse association of total PCBs with SHBG-bound testosterone (r=-0.29; p=0.04) (4). Similarly, in a study of 257 adult (age 18-95 yrs.) male Akwesanse Mohawk Native Americans, total testosterone was significantly lower among males in the highest tertile of serum total PCBs concentration compared to the lowest (OR=0.17; CI not reported) after adjusting for age, BMI, total lipids, HCB, DDE, and Mirex, but testosterone binding to SHBG was not assessed (30). This study also found significant inverse associations of total testosterone with 4 of 5 PCB groupings (mon-ortho, di-ortho, tri-tetra-ortho, dioxin-like TEQs) and 4 of 9 individual PCB congeners (PCB 74, PCB 99, PCB 153, PCB 206). A third study of 101 young (age 20-40 yrs.) Flemish males demonstrated a 7.1% decrease (-0.5%, -13.2%; p=0.04) in total testosterone and a 6.8% decrease in free testosterone (-0.4%, -12.7%; p=0.04) with a 2-fold increase in dioxin-like TEQs (CALUX-TEQs) (28). A study of 341 males (age 18-51 yrs.) attending a US infertility clinic demonstrated marginally significant ($p<0.10$) inverse associations of anti-estrogenic PCBs with both SHBG and total testosterone, but not free testosterone, after adjusting for age, BMI, and total lipids, but since raw betas were presented with different transformations used for each variable in each model, estimates are not do not have a direct clinical interpretation (29). A study of 196 middle-aged and elderly Swedish males (age 48-82 yrs.) found no significant relationship between PCBs and total testosterone or SHBG (32). In a study of 277 healthy, non-obese, middle-aged males (age 45-69 yrs.) from the French West Indies, a 2-fold increase in PCB 153 was associated with a 5.4% increase (1.0%, 9.8%) in androstenedione, a metabolic precursor to testosterone (not to be confused with 3α-androstanediol, a metabolite of testosterone), but no associations of PCBs with total, free, or SHBG-bound testosterone (46).

5.4.2 Testosterone Metabolism

Testosterone has two paths of metabolism: androgenic and estrogenic. In the estrogenic path, testosterone is converted to estradiol via the steroidogenic enzyme aromatase. We observed no associations of PCBs with total, bound, or free estradiol. We observed a significant association of increasing PB-type inducer PCBs with elevated Total T: Total E2 ratio, but this was largely driven by the strong association of PB-type inducer PCBs with total testosterone. The estrogenic effects of PCBs have been widely studied in a toxicological setting, with several studies demonstrating that certain PCB congeners can bind to and activate estrogen receptors. Since there is substantial overlap between PCB congeners classified as estrogenic (8,9) and those classified as PB-type inducer (10), we would have expected to observe an estrogenic effect of PB-type inducer PCBs. However, the epidemiologic literature on the estrogenic effect of PCBs is more consistent with our findings

In a study of Illinois capacitor manufacturing workers, Persky et al. (33) found a marginally significant positive association of estrogenic PCBs with total estradiol after adjustment for age, BMI group, and total lipids while Rylander et al. (32) found significantly lower concentrations of estradiol in the 4th quartile of serum PCB-153 concentration compared to the 1st quartile. Emeville et al. (46) found no association of PCB-153 with estradiol or estrone sulfate, but a significant positive association of PCB-153 with estrone was observed. In contrast, Persky et al. (5) and Turyk et al (4) consistently found no association of PCBs with estrone sulfate in the Great Lakes Sport-caught Fish Consumer Cohort and a null association of PCB-153 with estradiol was also found in the pooled international study by Giwercman et al. (45).

In the androgenic path of testosterone metabolism, testosterone is primarily converted into dihydrotestosterone (DHT) via the steroidogenic enzyme 5α-reductase. DHT is then

converted via glucuronidation to androstanediol glucuronide (3α-ADG) prior to elimination. DHT is a naturally occurring androgen that is 2 to 3 times more potent than testosterone with respect to androgen receptor binding. Thus, conversion of testosterone to DHT via 5α-reductase is a metabolic pathway that results in increased androgenicity. However, to a lesser extent, testosterone can also be metabolized directly to 3α-ADG, but this is also a 5α-reduced metabolic path. There also exists a backdoor path through which 3α-ADG can be synthesized that bypasses both testosterone and DHT through pregnolone. While this is not an androgenic path, it is a 5α-reduced path and all paths through which 3α-ADG is synthesized includes a metabolic reduction by 5α-reductase. It is for this reason that 3α-ADG has been used as a measure of 5α-reductase activity (47).

We found inverse associations of nearly all PCB groupings with 3α-ADG in the youngest (20-39 yrs.) and the middle (40-59 yrs.) age groups that were of large magnitudes and statistically significant ($p<0.05$), while there was no association observed in the oldest age group (60-80 yrs.). The PCB groupings resulting in the strongest negative associations with 3α-ADG were PB-type inducer PCBs and total PCBs. Given the above mechanism of androgen metabolism, it is plausible that circulating levels of 3α-ADG could be reduced by PCBs either through inhibition of testosterone conversion to DHT by 5α-reductase or conversion of DHT to 3α-ADG by glucuronidation. Only the former mechanism suggests an impact of PCB exposure on decreased 5α-reductase activity in males, but without a marker of glucuronidation activity, results are interpreted under the assumption that 5α-reductase activity is the primary mechanism of action.

The associations of PCBs with 3α-ADG appear to be strongly modified by age, with large magnitudes in the youngest and middle age groups contrasting null associations in the oldest age

group, but these associations are largely dependent on a positive association of PCBs with testosterone in the oldest age group. Thus, the positive associations of PCBs on total testosterone may be offsetting the inverse effect of PCBs on 3α-ADG, as suggested by the consistent associations of PB-type inducer PCBs and total PCBs on the total testosterone-standardized measure of 3α-ADG, the 3α-ADG to total T ratio. As a brief summary, inverse associations of PCBs with 3α-ADG were:

- Consistent across all PCB groupings, though strongest in PB-type inducer PCBs and total PCBs, and
- Offset by PCB-associated elevations in total testosterone in the oldest age group, but consistent across age groups after accounting for PCB associations with total testosterone

To the best of our knowledge, this is the first study exploring associations of PCBs with 3α-ADG. While 3α-ADG is not commonly explored as an outcome in hormone research studies, it is a biomarker that provides insight on androgen activity and metabolism. Although relationships between PCBs and 3α-ADG have not been explored in the past, a single study by Emeville et al. (46) found no association of PCB-153 with DHT. However, the toxicological literature provides some support PCBs inhibition of 5α-reductase activity. Two different studies of female rats and their male offspring exposed the male offspring to PCBs through the milk of mothers that were orally administered PCBs at varying doses. After sacrificing the male offspring and isolating samples of the Leydig cells of the testicles, both studies found a significant reduction in Leydig cell mRNA and protein expressions of 5α-reductase (48,49). Similarly, a study of the human prostate cancer cell line LNCaP found that LNCaP cells exposed to di-orthro dioxin-line PCBs 126 and 77 showed decreased androgen-dependent PSA and decreased 5α-reductase (50).

5.4.3 Summary

In summary, PB-type inducer PCBs and total PCBs were associated with elevated circulating SHBG in all age groups, but elevated total T and SHBG-bound T in only the oldest age group. In the oldest age group, the association of PB-type inducer PCBs with elevated total T completely explained the observed association of PB-type inducer PCBs with SHBG, but the association remained significant in the youngest age group. This suggests that PCBs may influence SHBG through disruption of the estrogen-androgen balance but may be both directly and indirectly dependent on age.

In addition, total PCBs, total TEQs, mixed-type inducers, and PB-type inducer PCBs were all associated with less circulating 3α-ADG, and these associations were highly dependent on concentrations of circulating total testosterone, but independent of age. This suggests that higher serum concentrations of PCBs may reduce 5α-reductase activity in androgen metabolism, which has implications toward the intermediate DHT as well as total androgen load. However, due to inconsistencies in relationships between androgens and 3α-androstanediol, the extent to which 3α-ADG reflects DHT or total androgens is not clear.

5.4.4 Strengths and Limitations

As with any study, the implications of this study are made with considerations of its limitations. First, this study uses a cross-sectional design. Since we cannot determine if PCB exposure occurred prior to measurement of hormones, there is added difficulty around making causal inferences. However, reverse causality is unlikely since, due to the long biological half-lives of PCBs, measures of serum PCBs reflect past as well as current exposure while measures of sex hormones reflect current sex hormone homeostasis.

With that said, another limitation of this study is that serum PCBs measured at a single time point do not provide any information on changes in PCB exposure and body burden over time. A participant presenting with low serum PCB concentrations at the time of this study may have had higher concentrations in the past. This is a common concern with environmental pollutants with shorter half-lives, such as phthalates, but the slow elimination of PCBs from the body results in low variability in body burden over longer time intervals. Furthermore, while PCBs have declined overall in the environment and in the population, the primary route of exposure to PCBs is through diet and as long as adiposity remains stable, PCBs would be stored at a constant rate influenced only by their elimination rates. Thus, the only likely scenario for a serum PCB measure not reflecting past exposure would be a drastic change in diet or a drastic weight gain/loss around the time the sample was taken.

Another limitation of this study is use of a dataset with missing data on serum PCB concentrations. For various reasons, concentrations of certain PCBs were missing in some participants. To overcome this limitation, we used advanced imputation techniques to estimate missing PCB concentrations and recover observed data on other important variables that would otherwise have been discarded. However, it should be noted that while the methods for imputation have limitations of their own, (explained in detail in the supplementary material), the benefits outweigh the risks of analyzing and interpreting highly missing data when data is missing at random.

The implications of this study speak to potential effects of PCBs on androgen metabolism and specifically on 5α-reductase activity. However, this study is limited to analysis of hormones measured by NHANES in the first two cycles, which did not include DHT. Without a direct measure of DHT, it was not possible to make the connection between 3α-ADG and DHT since

there are two other pathways through which 3α-ADG can be synthesized, one of which bypasses androgens entirely. Furthermore, 3α-ADG is a metabolite of DHT through a pathway which involves two metabolic processes: DHT reduction by 5α-reductase and glucuronidation. Previous toxicological studies of PCB effects on thyroid hormones have suggested that reduction in plasma thyroxine (T4) may be related to an increase in the enzyme that catalyzes glucuronidation of T4 (51). Thus, it is possible that the observed results may, at least in part, reflect PCB influence on glucuronidation. We were not able to ascertain whether the observed changes in 3α-ADG were due to action on 5α-reductase or glucuronidation, but any potential effects of PCBs on the glucuronidation pathway should be minimized since 3α-androstanediol (3α-ADiol) circulates mostly in its glucuronidated form, 3α-ADG (52).

Other hormones that were not directly measured are free and SHBG-bound fractions of testosterone or estradiol. We were able to estimate these fractions using information on total testosterone, SHBG, and serum albumin, though there is some controversy over this method since it does not account for SHBG-testosterone dimers.

Lastly, we explored multiple associations using several PCB grouping techniques and several hormone outcome measures. We chose to group PCBs by known toxicological mechanisms, but future investigations may employ alternative developing methodology for mixtures analysis to validate these groupings. Furthermore, we used several hormone measures that all reflect impacts on different and equally important pathways of sex hormone metabolism. Many comparisons were made without adjustment of p-values for multiple comparisons, which increases the risk of a false positive result. However, given the exploratory nature of this study, the decision was made to interpret p-values without adjustment for multiple comparisons to reduce the risk of false negative results.

Despite many limitations in the data and analysis, limitations were addressed, and the strengths outweigh the limitations. This study used a large sample, given the resources required to perform such intricate measures of both PCBs and sex hormones. This study also used advanced imputation methods to recover missing data and increase the power of the study. Finally, we performed a thorough analysis of multiple PCB groupings, each of which have been thought to act on a different biochemical pathway, and used multiple sex hormone endpoints, each of which are involved in a different stage in the metabolism of sex hormones.

5.5. Conclusions

To the best of our knowledge, this is the first study to demonstrate a relationship between PCBs and reduced testosterone metabolism through 5α-reductase activity. With an increasing body of literature on environmental chemicals and their potential impacts on androgen-sensitive chronic diseases such as coronary heart disease and prostate cancer, these results not only provide supporting evidence for those associations, but also a potentially unknown modifier of current intervention strategies that may need to be considered by physicians before making decisions on treatment of androgen-sensitive chronic diseases. Further research in the endocrine disrupting properties of environmental pollutants should focus not only on concentrations of hormones, but also on measures of hormone metabolism so that a specific biochemical pathway can be identified and potentially intervened upon.

Cited Literature

1. Hopf NB, Ruder AM, Waters MA, Succop P. Concentration-dependent half-lives of polychlorinated biphenyl in sera from an occupational cohort. *Chemosphere*. 2013;91(2):172-178.

2. Knutsen HK, Kvalem HE, Haugen M, et al. Sex, BMI and age in addition to dietary intakes influence blood concentrations and congener profiles of dioxins and PCBs. *Molecular nutrition & food research*. 2011;55(5):772-782.

3. Malisch R, Kotz A. Dioxins and PCBs in feed and food—review from European perspective. *Sci Total Environ*. 2014;491:2-10.

4. Turyk ME, Anderson HA, Freels S, et al. Associations of organochlorines with endogenous hormones in male Great Lakes fish consumers and nonconsumers. *Environ Res*. 2006;102(3):299-307.

5. Persky V, Turyk M, Anderson HA, et al. The effects of PCB exposure and fish consumption on endogenous hormones. *Environ Health Perspect*. 2001;109(12):1275-1283.

6. Hagmar L. Polychlorinated biphenyls and thyroid status in humans: A review. *Thyroid*. 2003;13(11):1021-1028.

7. Van den Berg M, Birnbaum LS, Denison M, et al. The 2005 world health organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. *Toxicol Sci*. 2006;93(2):223-241.

8. Wolff MS, Camann D, Gammon M, Stellman SD. Proposed PCB congener groupings for epidemiological studies. *Environ Health Perspect*. 1997;105(1):13-14.

9. Cooke PS, Sato T, Buchanan DL, Robertson L, Hansen L. Disruption of steroid hormone signaling by PCBs. *PCBs Recent Advances in Environmental Toxicology and Health Effects*. 2001:257-263.

10. Warner J, Osuch JR, Karmaus W, et al. Common classification schemes for PCB congeners and the gene expression of CYP17, CYP19, ESR1 and ESR2. *Sci Total Environ*. 2012;414:81-89.

11. McFarland VA, Clarke JU. Environmental occurrence, abundance, and potential toxicity of polychlorinated biphenyl congeners: Considerations for a congener-specific analysis. *Environ Health Perspect*. 1989;81:225-239.

12. Hansen LG. Stepping backward to improve assessment of PCB congener toxicities. *Environ Health Perspect*. 1998;106 Suppl 1:171-189.

13. Jansen HT, Cooke PS, Porcelli J, Liu T, Hansen LG. Estrogenic and antiestrogenic actions of PCBs in the female rat: In vitro and in vivo studies. *Reproductive Toxicology*. 1993;7(3):237-248.

14. Hany J, Lilienthal H, Sarasin A, et al. Developmental exposure of rats to a reconstituted PCB mixture or aroclor 1254: Effects on organ weights, aromatase activity, sex hormone levels, and sweet preference behavior. *Toxicol Appl Pharmacol*. 1999;158(3):231-243.

15. Negri-Cesi P, Colciago A, Pravettoni A, Casati L, Conti L, Celotti F. Sexual differentiation of the rodent hypothalamus: Hormonal and environmental influences. *J Steroid Biochem Mol Biol*. 2008;109(3-5):294-299.

16. Govarts E, Nieuwenhuijsen M, Schoeters G, et al. Birth weight and prenatal exposure to polychlorinated biphenyls (PCBs) and dichlorodiphenyldichloroethylene (DDE): A meta-analysis within 12 European birth cohorts. *Environ Health Perspect*. 2012;120(2):162-170.

17. Vafeiadi M, Agramunt S, Papadopoulou E, et al. In utero exposure to dioxins and dioxin-like compounds and anogenital distance in newborns and infants. *Environ Health Perspect*. 2013;121(1):125-130.

18. Neugebauer J, Wittsiepe J, Kasper-Sonnenberg M, Schöneck N, Schölmerich A, Wilhelm M. The influence of low level pre-and perinatal exposure to PCDD/fs, PCBs, and lead on attention performance and attention-related behavior among German school-aged children: Results from the Duisburg birth cohort study. *Int J Hyg Environ Health*. 2015;218(1):153-162.

19. Winneke G, Ranft U, Wittsiepe J, et al. Behavioral sexual dimorphism in school-age children and early developmental exposure to dioxins and PCBs: A follow-up study of the Duisburg cohort. *Environ Health Perspect*. 2014;122(3):292-298.

20. Burns JS, Lee MM, Williams PL, et al. Associations of peripubertal serum dioxin and polychlorinated biphenyl concentrations with pubertal timing among Russian boys. *Environ Health Perspect*. 2016;124(11):1801-1807.

21. Denham M, Schell LM, Deane G, et al. Relationship of lead, mercury, mirex, dichlorodiphenyldichloroethylene, hexachlorobenzene, and polychlorinated biphenyls to timing of menarche among Akwesasne Mohawk girls. *Pediatrics*. 2005;115(2):e127-34.

22. Zhang J, Huang Y, Wang X, Lin K, Wu K. Environmental polychlorinated biphenyl exposure and breast cancer risk: A meta-analysis of observational studies. *PLoS One*. 2015;10(11):e0142513.

23. Prince MM, Hein MJ, Ruder AM, Waters MA, Laber PA, Whelan EA. Update: Cohort mortality study of workers highly exposed to polychlorinated biphenyls (PCBs) during the manufacture of electrical capacitors, 1940-1998. *Environ Health*. 2006;5:13.

24. Zong G, Valvi D, Coull B, et al. Persistent organic pollutants and risk of type 2 diabetes: A prospective investigation among middle-aged women in nurses' health study II. *Environ Int*. 2018;114:334-342.

25. Turyk M, Anderson H, Knobeloch L, Imm P, Persky V. Organochlorine exposure and incidence of diabetes in a cohort of Great Lakes sport fish consumers. *Environ Health Perspect*. 2009;117(7):1076-1082.

26. Bergkvist C, Berglund M, Glynn A, Wolk A, Åkesson A. Dietary exposure to polychlorinated biphenyls and risk of myocardial infarction–A population-based prospective cohort study. *Int J Cardiol*. 2015.

27. Blackowicz M, Persky V, Freels S, Anderson H, Turyk M. Cardiovascular disease incidence and exposure to persistent organic pollutant in Great Lakes fish consumers. . 2016.

28. Dhooge W, van Larebeke N, Koppen G, et al. Serum dioxin-like activity is associated with reproductive parameters in young men from the general Flemish population. *Environ Health Perspect*. 2006;114(11):1670-1676.

29. Ferguson KK, Hauser R, Altshul L, Meeker JD. Serum concentrations of p, p′-DDE, HCB, PCBs and reproductive hormones among men of reproductive age. *Reproductive Toxicology*. 2012;34(3):429-435.

30. Goncharov A, Rej R, Negoita S, et al. Lower serum testosterone associated with elevated polychlorinated biphenyl concentrations in native American men. *Environ Health Perspect*. 2009;117(9):1454-1460.

31. Richthoff J, Rylander L, Jonsson BA, et al. Serum levels of 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153) in relation to markers of reproductive function in young males from the general Swedish population. *Environ Health Perspect*. 2003;111(4):409-413.

32. Rylander L, Wallin E, Jönssson BA, Stridsberg M, Erfurth EM, Hagmar L. Associations between CB-153 and p, p′-DDE and hormone levels in serum in middle-aged and elderly men. *Chemosphere*. 2006;65(3):375-381.

33. Persky V, Piorkowski J, Turyk M, et al. Polychlorinated biphenyl exposure, diabetes and endogenous hormones: A cross-sectional study in men previously employed at a capacitor manufacturing plant. *Environ Health*. 2012;11(1):57.

34. Liu S, Sun Q. Sex differences, endogenous sex-hormone hormones, sex-hormone binding globulin, and exogenous disruptors in diabetes and related metabolic outcomes. *Journal of diabetes*. 2018;10(6):428-441.

35. Kim C, Halter JB. Endogenous sex hormones, metabolic syndrome, and diabetes in men and women. *Curr Cardiol Rep*. 2014;16(4):467.

36. Ding EL, Song Y, Malik VS, Liu S. Sex differences of endogenous sex hormones and risk of type 2 diabetes: A systematic review and meta-analysis. *JAMA*. 2006;295(11):1288-1299.

37. Clegg D, Hevener AL, Moreau KL, et al. Sex hormones and cardiometabolic health: Role of estrogen and estrogen receptors. *Endocrinology*. 2017;158(5):1095-1105.

38. Kloner RA, Carson C, Dobs A, Kopecky S, Mohler ER. Testosterone and cardiovascular disease. *J Am Coll Cardiol*. 2016;67(5):545-557.

39. El Khoudary SR, Shields KJ, Janssen I, et al. Cardiovascular fat, menopause, and sex hormones in women: The SWAN cardiovascular fat ancillary study. *The Journal of Clinical Endocrinology & Metabolism*. 2015;100(9):3304-3312.

40. Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS), ed. *National health and nutrition examination laboratory procedures manual.* Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention; 2000.

41. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. *The Journal of Clinical Endocrinology & Metabolism*. 1999;84(10):3666-3672.

42. Bernert JT, Turner WE, Patterson DG, Needham LL. Calculation of serum "total lipid" concentrations for the adjustment of persistent organohalogen toxicant measurements in human samples. *Chemosphere*. 2007;68(5):824-831.

43. Van Buuren S, Groothuis-Oudshoorn K. Mice: Multivariate imputation by chained equations in R. *Journal of statistical software*. 2010:1-68.

44. Uwimana E, Ruiz P, Li X, Lehmler H. Human cyp2a6, cyp2b6 and cyp2e1 atropselectively metabolize polychlorinated biphenyls to hydroxylated metabolites. *Environ Sci Technol*. 2019.

45. Giwercman AH, Rignell-Hydbom A, Toft G, et al. Reproductive hormone levels in men exposed to persistent organohalogen pollutants: A study of Inuit and three European cohorts. *Environ Health Perspect*. 2006;114(9):1348-1353.

46. Emeville E, Giton F, Giusti A, et al. Persistent organochlorine pollutants with endocrine activity and blood steroid hormone levels in middle-aged men. *PloS one*. 2013;8(6):e66460.

47. Hsing AW, Reichardt JK, Stanczyk FZ. Hormones and prostate cancer: Current perspectives and future directions. *Prostate*. 2002;52(3):213-235.

48. Kumar SV, Bhattacharya S. In vitro toxicity of mercury, cadmium, and arsenic to platelet aggregation: Influence of adenylate cyclase and phosphodiesterase activity. *In Vitro & Molecular Toxicology: A Journal of Basic and Applied Research*. 2000;13(2):137-144.

49. Yokoyama M, Origasa H, Matsuzaki M, et al. Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): A randomised open-label, blinded endpoint analysis. *The Lancet*. 2007;369(9567):1090-1098.

50. Endo F, Monsees TK, Akaza H, Schill W, Pflieger-Bruss S. Effects of single non-ortho, mono-ortho, and di-ortho chlorinated biphenyls on cell functions and proliferation of the human prostatic carcinoma cell line, LNCaP. *Reproductive toxicology*. 2003;17(2):229-236.

51. Barter RA, Klaassen CD. UDP-glucuronosyltransferase inducers reduce thyroid hormone levels in rats by an extrathyroidal mechanism. *Toxicol Appl Pharmacol*. 1992;113(1):36-42.

52. Ross RK, Bernstein L, Pike M, et al. 5-alpha-reductase activity and risk of prostate cancer among Japanese and US white and black males. *The Lancet*. 1992;339(8798):887-889.

6. FINAL CONCLUSIONS

6.1. Summary of Findings

This study aimed to (1) explore longitudinal relationships between fish consumption, POPs, and CHD-related outcomes in the GLSCF Consumer Cohort, (2) explore associations over time between POPs and endogenous steroid hormones in the GLSCF Consumer Cohort, and (3) explore cross-sectional associations between POPs and endogenous steroid sex hormones in using data from the NHANES.

The results of the first study demonstrated a significantly increased risk of self-reported physician diagnosis of CHD with increasing serum concentrations of PCB congeners which are known phenobarbital-type (PB-type) inducers of metabolic cytochrome P450 enzymes of the 2A and 2B family. Specifically, after adjusting for known and suspected confounders, there was a 72% increase in risk of CHD for each doubling of serum concentrations of PB-type PCB congeners ($p = 0.0294$).

In the second study, PCBs were also associated with steroid sex hormones, which may potentially be a pathway through which PCBs increase risk of CHD. After excluding participants with liver disease, inverse associations of PCBs with SHBG (-16.5% for each doubling of PB-type PCBs, p=0.03) and SHBG-bound testosterone (-17.9%, p=0.02), paired with associations in the opposite direction for free testosterone (9.7%, p=0.07), are consistent with a PCB-induced inhibition of SHBG synthesis resulting in reduced binding of total testosterone to SHBG and leaving a larger proportion of testosterone circulating freely in the blood.. We found a similar significant inverse association of DDE with SHBG-bound testosterone (-22.9%, p=.0.3), but limited evidence for an impact of DDE on SHBG (-8%, p=0.14) and free testosterone (2.8%, p=0.45). However, there were no associations with PCBs or DDE with total testosterone and the

strength and precision of the observed estimates of association with SHBG-bound and free testosterone varied depending on the compound and the PCB enzyme induction properties, with the strongest associations consistently found for PB-type inducer PCBs, and the weakest with MC-type inducers or mixed inducers.

In contrast, in the cross-sectional study using data from NHANES, PB-type PCBs were associated with reduced 5α-reductase activity in androgen metabolism, as measured by 3a-androstanediol glucuronide in men. Specifically, in the youngest age group, we found a 17% reduction in 3α-ADG for each 50% increase in PB-type PCBs (p=0.001) and this association became weaker with age, resulting in no observed association in the oldest age group. Since we also observed a 19% increase in total testosterone in only the oldest age group (p=0.046), we used a testosterone-standardized estimate of 3α-ADG (3α-ADG: total testosterone ratio) to provide additional insight on 5α-reductase activity. After standardizing to total testosterone, the inverse association of PB-type PCBs with 3α-ADG was consistent across all age groups, indicating an overall reduction in 5α-reductase activity with increasing PB-type PCBs. Furthermore, PB-type PCBs were associated with an 8% increase in SHBG across all age groups (p=0.009), though this association was potentially mediated by the increase in total testosterone in the oldest age group.

The complexity of hormone metabolism makes it difficult to draw sound conclusions, but 5α-reductase is well understood to be a key enzyme in androgen metabolism. This enzyme is essential for elimination of testosterone and for conversion of testosterone to the more potent DHT. Reduction of 5α-reductase activity by PCBs could potentially increase risk of prostate cancer and reduce the effectiveness of common treatments for prostate cancer. Furthermore, reduced 3α-ADG may have implications toward lower androgen activity, which has been

associated with many chronic health conditions, including CHD. The strongest associations were observed in the youngest age group, which presents young men as a potentially high risk group for PCB exposure with respect to CHD related outcomes not only because they have a longer potential duration of exposed, but also an earlier start to atherosclerotic progression, which is liked to earlier onset of CHD and earlier risk of death from myocardial infarction.

While all three studies followed the same guidelines for classification of PCB congeners according to their enzyme induction activity, there were differences in the laboratory methodology between the Great Lakes study and the NHANES study and also between survey years for both studies, which is likely to have introduced exposure misclassification. While this misclassification is likely not differential with respect to any outcome of interest or any unknown confounder (i.e. missing at random), it may introduce excess variability which results in bias toward the null associations.

Due to the complex nature of cardiometabolic diseases, caution was taken in exploring associations of POPs with CHD and sex hormone disruption in the presence of comorbid diabetes. Since POPs are strongly associated with diabetes, and diabetes is known to play a role in CHD, hormone binding, and hormone metabolism, there is potential for effect modification, mediation, and confounding by diabetes in these associations. After careful consideration of the role of diabetes, these findings suggest that diabetes does not impact the observed results and the role of POPs in increasing risk of CHD and steroid hormone disruption may be independent of diabetes.

While both the Great Lakes and NHANES studies showed strong associations between PB-type PCBs and SHBG, the associations were in opposite directions. It is unclear why we observed such a large inconsistency in associations of PCBs with SHBG, but with a large

potential for measurement error of both PCBs and SHBG as well as potential confounding by unmeasured exposures, including methyl mercury, a common contaminant in fish, it is possible that one or both of these estimates may be impacted by bias resulting from measurement error and/or confounding. This is particularly impactful in the Great Lakes study where study participants were intentionally selected based upon their suspected exposure to contaminated fish and lack of previous diagnosis of diabetes.

6.2. Significance and Contribution to the Field

The Great Lakes Sport-caught Fish Consumer cohort is one of the longest ongoing studies exploring long-term effects of consuming contaminated fish on cardiovascular/metabolic outcomes and endocrine disruption and this cohort provides a unique opportunity to study these associations in a high-risk population of Great Lakes sport-caught fish consumers along with locally matched non-consumer referents. The literature around organochlorines exposure, incidence of CHD-related outcomes, and steroid hormone metabolism is both limited and inconsistent with respect to results as well as the methods used to ascertain exposure, outcomes, and known confounders.

Inconsistencies are often due to limited resources to measure blood biomarkers over a long period of time in a sample of the general population that is large enough to detect meaningful differences in hormones or organochlorines. The study included resource intensive direct blood measures of serum DDE and PCBs, steroid hormones, and other important biomarkers, such as hemoglobin A1c, cholesterol, and triglycerides. Furthermore, the study included extensive data on fish consumption, a major confounder of these association as they are

a source of both potentially toxic environmental pollutants as well as cardioprotective omega-3 polyunsaturated fatty acids.

In addition to inconsistencies in methods and results, most of the existing literature on these topics have a cross-sectional or ecological design, which prevents researchers from applying causal inference. This study contributed greatly to the literature as a study documenting long-term exposure to PCBs at general population levels with long-term follow up that is sufficient to detect differences in risk of cardiovascular outcomes that develop much later in life, as well as a plausible and scientifically supported mechanism of action for PCBs increasing risk of CHD.

6.3. Recommendations for Future Research

Future research would greatly benefit from improvements upon the currently established methods for grouping of PCBs. While it is useful to identify and take the sum of PCB concentrations which have similar mechanisms of action, this does not address issues of potency nor does it address issues of multiple mechanisms. Furthermore, new methods should focus on exploring the potential impacts of multiple high correlated mixtures of exposures such as various PCB congeners, dioxins/furans, organochlorine pesticides, methyl-mercury, phthalates, polybrominated diphenyl ethers, polyfluorinated organic compounds, and more.

Furthermore, the results from this study could be greatly improved upon with more detail on sex hormone intermediates, such as DHT, to better understand the complex interactions between PCBs and hormones, as well as more detail around other plausible mechanisms of action for PCBs on CHD-related outcomes, such as lipid peroxidation, number/thickness of atherosclerotic plaques, and markers of inflammation. Finally, future studies may be able to tell a

more complete story of the complexities of these relationships by using advanced structural equation models or Bayesian methods to better understand the roles of circulating steroid sex hormones, diabetes, thyroid disease, liver disease, and obesity in the associations of PCBs with CHD.

State public health agencies should continue to promote consumption of fatty fish for improved heart health, since fatty fish are an excellent source of omega 3 polyunsaturated fatty acids and other nutrients, but it is also important to highlight in public fishing advisories that consuming fish from lakes known to be contaminated with PCBs may counteract the health benefits of fish consumption. Specifically, fish advisories should continue to suggest avoidance of large predatory fish of the Great Lakes, such as salmon, walleye, and trout, particularly from lakes with higher concentrations of PCBs, such as Lake Michigan, Lake Huron, and Lake Ontario.

6.4. Concluding Remarks

This study provides evidence that PCBs, and particularly PCB congeners that are known PB-type inducers of CYP2A and CYP2B, may increase risk of CHD and the increase in risk of CHD may, at least in part, be a result of modified androgen homeostasis in males. The difference in results according to PCB grouping method highlights the importance of developing new methods for assessing the impact of multiple exposures to environmental pollutants in the presence of highly correlated exposure mixtures.

APPENDICES

Appendix A

Imputation Methods (GLSCF Consumer Cohort)

Missing data was imputed using fully conditional specification of multiple imputations by chained equations (MICE) in the R package 'mice' (133) and all imputed data were assumed to be missing at random. All participants with at least one measurement of PCBs/DDE in 1996, 2001, or 2004 were included in the imputation. Binary variables were imputed using logistic regression (logreg), factor variables with more than 2 categories were imputed using polytomous logistic regression (polyreg), and continuous variables were imputed using predictive mean matching (ppm). PCB groupings and data transformations were done on the final post-imputation dataset. While this is a limitation of the imputation method, it was deemed necessary as the slight increase in bias in using "impute then transform" did not outweigh the added computational complexity of "transform then impute".

Missing data on all PCB grouping, demographic, clinical, and health history variables were imputed except outcome variables (i.e. diagnoses, time of diagnoses, serum hormones), and study design variables (i.e. follow-up survey indicators, follow-up survey dates). All variables, including those that were not imputed, were used as predictors in imputation models for imputed variables. Household/spousal couple identifier was used as a clustering predictor for imputation models. Variables were imputed in order of increasing missingness, starting variables having the least missing data (i.e. monotone). Each fully imputed dataset underwent 5 imputation iterations for a total of 20 fully imputed datasets. The 20 imputation sets were merged and exported in long form. All analyses were executed in SAS using PROC MIANALYZE.

1. Van Buuren S, Groothuis-Oudshoorn K. Mice: Multivariate imputation by chained equations in R. *Journal of statistical software*. 2010:1-68.

Appendix B

Imputation Methods (NHANES 1999-2002)

Missing data was imputed using multiple imputations by chained equations (MICE) in the R package 'mice' (133) and all imputed data were assumed to be missing at random. All participants from POPs subsample C in cycles A (1999-2000), B (2001-2002), and C (2003-2004) were included in the imputation. Binary variables were imputed using logistic regression (logreg), factor variables with more than 2 categories were imputed using polytomous logistic regression (polyreg), and continuous variables were imputed using predictive mean matching (ppm). Clustering by survey design parameters was accounted for by assigning 'Strata' as a class variable and 'PSU' as a random variable. Unique identifier 'SEQN' and survey weights were not used to inform imputations.

Variables were visited using monotone visit sequence over 5 iterations for a generation of 20 imputation datasets. Imputations were done separately for each age group (20-39, 40-59, 60-85) to account for complex interactions between PCBs, hormones, and age. The 20 imputation datasets (m1 through m20) for each age group were all combined into a single dataset and appended with the original unimputed dataset (m0). PCB groupings and data transformations were done on the final post-imputation dataset. While this is a limitation of the imputation method, it was deemed necessary as the slight increase in bias in using "impute then transform" did not outweigh the added computational complexity of "transform then impute".

1. Van Buuren S, Groothuis-Oudshoorn K. Mice: Multivariate imputation by chained equations in R. *Journal of statistical software*. 2010:1-68.

Appendix C

IRB Documentation – Great Lakes Fish Consumer Studies

Approval Notice
Continuing Review

November 19, 2019

Mary Ellen Turyk, PhD
Epidemiology and Biostatistics
Phone: (312) 355-4673 / Fax: (312) 996-0064

RE: **Protocol # 2010-0003**
"Diabetes and Persistent Organic Pollutants"

Dear Dr. Turyk:

Your application was reviewed and approved by the Expedited review process on November 19, 2019. You may now continue your research.

Please note the following information about your approved research protocol:

Protocol Approval Date: November 19, 2019
Approved Subject Enrollment #: 954 (limited to data analysis only)
Performance Sites: UIC, Wisconsin Department of Health Services
Sponsor: NIEHS - National Institute of Environmental Health Sciences, NIEHS, School of Public Health
Institutional Proposal (IP) #: 00454099,00342833,Not available
Grant/Contract No: 1 R21 ES030792-01,1R01ES028790-01,Not available
Grant/Contract Title: Endocrine disruption by perfluoroalkyl substances and mercury,Innovative Methodologic Advances for Mixtures Research in Epidemiology,Not available
Research Protocol(s):
a) Diabetes and Persistent Organic Pollutants, Version 11, 6/12/2019

Informed Consent(s):
a) Not applicable- research is 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.

Your research continues to meet the criteria for expedited review as defined in 45 CFR

UNIVERSITY OF ILLINOIS AT CHICAGO
Office for the Protection of Research Subjects
201 AOB (MC 672)
1737 West Polk Street
Chicago, Illinois 60612
Phone (312) 996-1711

Appendix C (Continued)

IRB Documentation – Great Lakes Fish Consumer Studies

46.110(b)(1) under the following specific categories:

Protocol reviewed under expedited review procedures [45 CFR 46.110] Category: 2, 5, 7

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 the policies of the UIC Human Subjects Protection Program (HSPP) and the guidance *Investigator Responsibilities.*

Please note that the UIC IRB has the prerogative and authority to ask further questions, seek additional information, require further conditions, 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 the OPRS office at (312) 996-1711 or me at (312) 413-9680. Please send any correspondence about this protocol to OPRS via OPRS Live.

Sincerely,

Jovana Ljuboje, MPA
Assistant Director, IRB # 1
Office for the Protection of Research Subjects

cc: Ronald C. Hershow, Epidemiology and Biostatistics, M/C 923
OVCR Administration, M/C 672

UNIVERSITY OF ILLINOIS AT CHICAGO
Office for the Protection of Research Subjects
207 AOB (MC 672)
1737 West Polk Street
Chicago, Illinois 60612
Phone (312) 996-1711

Appendix D

IRB Documentation – NHANES Study

Notice of Determination of Not Human Subject Research

April 2, 2018

Michael Blackowicz, MS
Epidemiology and Biostatistics
1603 W. Taylor
Phone: (708) 254-1198 / Fax: (312) 996-0064

RE: **Protocol # 2018-0355**
Serum PCBs and Circulating Sex Hormones

Sponsor:	None
PAF#:	Not applicable
Grant/Contract No:	Not applicable
Grant/Contract Title:	Not applicable

Dear Mr. Blackowicz:

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).

Specifically, the project is limited to accessing one of the public use datasets which OPRS has determined to not meet human subjects research. The project is accessing the dataset from National Center for Health Statistics, National Health and Nutrition Examination Survey (NHANES) from 1999-2002.

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.

VITA

Michael J. Blackowicz

Education

2013 – 2019	**Ph.D. Candidate,** ***Occupational and Environmental Epidemiology*** University of Illinois at Chicago, Chicago, IL Dissertation Topic: Persistent organic pollutants, circulating sex hormones, and coronary heart disease Focus: Environmental Epidemiology
2010 – 2013	**M.S.,** ***Biological Sciences*** Eastern Illinois University, Charleston, IL Thesis Topic: The effects of industrial biomasss gasification ash as a soil amendment on the earthworm *Eisenia fetida* Focus: Environmental Chemistry & Ecotoxicology
2005 – 2009	**B.S.,** ***Biological Sciences*** Northern Illinois University, DeKalb, IL Major Focus: General Biological Sciences Minor Focus: Chemistry

Research Publications and Manuscripts

1. **Blackowicz MJ**, Persky VW, Freels S, Anderson HA, Turyk ME (2020). Persistent organic pollutants and incident coronary heart disease in Great Lakes fish consumers. *Manuscript in preparation for 2020 submission.*
2. **Blackowicz MJ**, Persky VW, Sargis R, Freels S, Turyk ME (2020). Associations of PCBs with circulating sex hormones in men. *Manuscript in preparation for 2020 submission.*
3. Nagel E**, Blackowicz MJ.**, Sahr F, Jarrett, OD (2019). Impact of the Ebola epidemic on clinical outcomes of HIV-infected soldiers and their dependents in Sierra Leone. *International journal of STD & AIDS*, *30*(2), 106-112.
4. Christensen KY, Raymond M, **Blackowicz MJ**, Liu Y, Thompson BA, Anderson HA, Turyk M (2017) Perfluoroalkyl substances and fish consumption. *Environmental Research, 154, 145-151.*
5. **Blackowicz MJ**, Hryhorczuk DO, Rankin KM, Lewis DA, Haider D, Lanphear BP, Even, A. (2016). The impact of low-level lead toxicity on school performance among Hispanic subgroups in Chicago public schools. *International Journal of Environmental Research and Public Health, 13(8), 774.*

Grants & Awards

2012	Graduate Research/Creative Activity Award Eastern Illinois University Office of the Graduate School
2011	Illinois Wildlife Preservation Fund Small Grant Illinois Department of Natural Resources
2008	Undergraduate Research Apprenticeship Northern Illinois University Department of Biological Sciences

Research Presentations

1. **Blackowicz MJ**; Campbell A; Floden L; Hudgens S; Basch E (2019). Mediation by Progression of Treatment-related Differences in Patient Reported Outcomes (PROs) in Oncology. Research will be presented at: *The 2019 Joint Statistical Meeting; 2019 Jul 27-Aug 1; Denver, Colorado.*
2. **Blackowicz MJ**, Persky VW, Freels S, Anderson HA, Turyk ME (2016). Associations of persistent organic pollutants with incident coronary heart disease in a cohort of Great Lakes fish consumers. Research presented at: *28h Annual Conference for the International Society for Environmental Epidemiology (ISEE); 2016 Sep 1-4; Rome, Italy.*

Grants & Awards

2012	**Graduate Research/Creative Activity Award** Eastern Illinois University Office of the Graduate School, Charleston, IL
2011	**Illinois Wildlife Preservation Fund Small Grant** Illinois Department of Natural Resources, Springfield, IL
2008	**Undergraduate Research Apprenticeship** Northern Illinois University Department of Biological Sciences, DeKalb, IL

Professional Memberships

American Statistical Association (ASA)

International Society for Environmental Epidemiology (ISEE)

Teaching Experience

2017 **Course Developer, Epidemiology and Biostatisics**
School of Public Health, University of Illinois at Chicago, Chicago, IL
- Independently developed "Introduction to SAS" online supplementary video course

2014 – 2017 **Graduate Teaching Assistant, Epidemiology and Biostatisics**
School of Public Health, University of Illinois at Chicago, Chicago, IL
- (1) Introduction to Epidemiology Concepts and Methods
- (2) Analytic Methods in Public Health
- (3) Intermediate Epidemiologic Methods
- (4) Advanced Epidemiologic Methods

2010 – 2013 **Graduate Teaching Assistant, Biological Sciences**
School of Public Health, University of Illinois at Chicago, Chicago, IL
- (1) Animal Diversity
- (2) Biology Forum
- (3) Ecology
- (4) Resource Management & Environmental Assessment

Research Experience

2013 – 2018 **NIOSH Occupational and Environmental Epidemiology Fellow**
University of Illinois at Chicago School of Public Health and the National Institute for Occupational Safety and Health

2017 **Graduate Research Assistant, Epidemiology and Biostatistics**
School of Public Health, University of Illinois at Chicago, Chicago, IL
- Developed functional datasets from laboratory and survey results
- Performed univariate and multivariable analysis for associations of chemical exposures and several continuous biomarker indicators of cardio-metabolic health