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In utero and childhood DDT, DDE, PBDE and PCBs exposure and sex hormones in adolescent boys: The CHAMACOS study

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ABSTRACT

Dichlorodiphenyltrichloroethane (DDT), polybrominated diphenyl ether (PBDE) flame retardants, and polychlorinated biphenyls (PCBs) are believed to be endocrine-disrupting chemicals (EDCs) in humans and animals. The purpose of this study is to examine the relationship of *in utero* and childhood exposure to these purported EDCs and reproductive hormones in adolescent boys who participated in CHAMACOS, an ongoing birth cohort in California's Salinas Valley. We measured *o,p'*- and *p,p'*-DDT, *p,p'*-DDE, PBDEs and PCBs in serum collected from mothers during pregnancy or at delivery and from their sons at 9 years. We measured concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and total testosterone (T) from 234 of their sons at 12 years. In adjusted models, we found that a 10-fold increase in maternal prenatal serum concentrations of BDE-153 was associated with a 22.2% increase (95% CI: 1.0, 47.9) in FSH, a 96.6% increase (95% CI: 35.7, 184.7) in LH, and a 92.4% increase (95% CI: 20.9, 206.2) increase in T. Similarly, BDE-100 concentrations were associated with increases in boys' LH levels. A 10-fold increase in total prenatal ΣPCBs was associated with a 64.5% increase (95% CI: 8.6, 149.0) in FSH, primarily driven by non- dioxin-like congeners. Boys' hormone levels were only marginally associated with prenatal DDT or DDE in primary models, but when boys' Tanner stage at age 12 was added to models, prenatal maternal DDT levels were associated with decreases in LH (adjusted percent change per 10-fold increase = −18.5%, 95% CI: −29.8, −5.4) and T (percent change = −18.2%, 95% CI: −30.2, −4.2) and DDE with LH (percent change = −18.3%, 95% CI: −32.9, −0.6). Exposures measured in the children's serum at 9 years also showed associations between BDE-153 and ΣPCBs. However, there is evidence that these associations appear to be mediated by child BMI. This study suggests associations on male hormones of 12 year old boys related to exposure to certain EDC exposure prenatally. The implications on future reproductive function in puberty and adulthood should be determined.

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1. Introduction

Chemicals such as dichlorodiphenyltrichloroethane (DDT), polybrominated diphenyl ethers (PBDEs), and polychlorinated biphenyls (PCBs), are hypothesized to be endocrine disruptors – chemicals that mimic, block, or interfere with the body's natural hormones (Chiabotto et al., 2006; Diamanti-Kandarakis et al., 2009; Massart et al., 2006). DDT, an organochlorine pesticide, has not been used in the United States (US) since 1972 and in Mexico since 2000 (Blanco-Munoz et al., 2016); nonetheless, most US residents have measurable levels of the its metabolite, dichlorodiphenyldichloroethylene (DDE) (CDC, 2013). The commercial PBDE product penta-BDE (consisting of the congeners BDEs

Abbreviations: BMI, body mass index; CHAM1, CHAMACOS 1 (birth cohort); CHAM2, CHAMACOS 2 (enrolled at age 9 and followed prospectively); CV, coefficients of variation; DDT, dichlorodiphenyltrichloroet hane; DDE, dichlorodiphenyldichloroeth ylene; FSH, follicle-stimulating hormone; GM, geometric mean; GSD, gemoetric standard deviation; kg/m^2, Kilogram per meter squared; LH, luteinizing hormone; LLOQ, lower limit of quantification; LOD, limits of detection; mIU/ml, milli international units per milliliter; ng/dL, nanograms per deciliter; PBDE, polybrominated diphenyl ethers; PCB, polychlorinated biphenyls; pg, picograms; T, total testosterone; TB, Technical Bulletin; US, United States; ≥, greater than or equal to; <, less than.

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47, 99, 100, and 153) was added to foam-based furniture, textiles, and other consumer products until 2005, but continues to leach out from older household items. PCBs were used in the United States in dielectric and coolant fluids until 1979. These three chemical groups have long half-lives, are lipophilic, and bioaccumulate in the environment and in living organisms (SCPOP, 2016a; UNEP, 2001). They readily cross the placenta and reach the developing fetus (Vizcaino et al., 2014). Under the Stockholm Convention, PCBs and penta-BDE have been eliminated, and DDT use has been restricted to disease vector control (SCPOP, 2016b; UNEP, 2001). Although these chemicals are hypothesized to affect hormones, there are few human studies. Recent evidence suggests that age of pubertal onset in boys, defined by testicular enlargement (gonadarche) and the appearance of pubic hair (pubarche), may also be decreasing, and exposure to endocrine disruptors has been implicated (Herman-Giddens et al., 2001; Herman-Giddens, 2006).

DDT compounds, such as the isomers *p,p′*-DDT and *o,p′*-DDT that are found in technical grade DDT, are known to be at least mildly estrogenic, and *p,p′* DDE inhibits androgens from binding to their receptors (Kelce et al., 1995). A study of 120 Akwesasne Mohawk boys (aged 10–17 years) reported that concurrent measurements of *p,p′*-DDT were not associated with testosterone (T) levels, although *p,p′*-DDE levels were positively but not significantly associated (Schell et al., 2014). The one previous study that examined the association of *in utero* exposure to DDT/DDE on the hormones of adolescent boys found no association between maternal prenatal levels during a time of peak use in the US and their sons' T levels at ages 10–17 years (n = 260) (Gladen et al., 2004).

In estrogen receptor binding assays, lower-brominated PBDEs (e.g. BDE-28, −47 and −100) exhibit estrogenic activity (Dang et al., 2007; Meerts et al., 2001), while higher-brominated congeners (e.g. BDE-153 and −190) display anti-estrogenic properties (Meerts et al., 2001). The penta-BDE mixture also exhibits anti-androgenic activity in androgen receptor binding assays (Harju et al., 2007; Stoker et al., 2005). PBDEs have been found to be anti-androgenic *in vivo* in male rodent models, with studies of *in utero* BDE-99 exposure resulting in significant decreases in T in male offspring and a slight acceleration in onset of puberty (preputial separation) in low-dose males (Lilienthal et al., 2006). However, peripubertal penta-BDE exposure caused delayed preputial separation in male rodents (Stoker et al., 2004). No previous studies have examined the relationship in humans of *in utero* PBDE exposure on sex hormone levels, but significant inverse associations have been reported between penta-BDEs in house dust and FSH, LH, and free androgen index (molar ratio of total T to sex hormone-binding globulin) in a study of 24 men recruited from an infertility clinic (Meeker et al., 2009), but only the association with FSH persisted in the expanded sample of 62 men (Johnson et al., 2013).

PCB exposure studies have demonstrated conflicting relationships with male hormones in both animal (Wakui et al., 2010; Xiao et al., 2011; Xu et al., 2013; Zhu et al., 2013) and human studies. For example, in a cohort of boys with higher prenatal exposure to PCBs during the Yucheng incident in 1978–79 (n = 47) and unexposed controls (n = 49), exposed boys who were at puberty (≥13 years old) had lower T levels and higher estradiol and FSH levels (non-significant) compared to controls, but there was no difference in boys <13 years; there were also no differences in LH levels (Hsu et al., 2005). Conversely, in a much larger study of the Faroe Islands birth cohort, PCBs in cord blood were inversely related to serum concentrations of T and LH in 14-year old boys (n = 433) (Grandjean et al., 2012).

In the present study, we determine the relationship between prenatal and childhood serum concentrations of DDT/DDE, penta-BDEs, and PCBs on the sex hormones of 12-year old boys participating in the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) birth cohort study. We hypothesize that early life exposure to EDCs will alter sex hormones of adolescent boys, although the direction of change is not apparent from prior literature.

2. Methods

2.1. Study participants

Pregnant women were recruited between October 1999 and October 2000 from community clinics. Women were eligible for participation if they were ≥18 years old, <20 weeks gestation, Spanish- or English-speaking, qualified for low-income health insurance, and planned to deliver at the public hospital. A total of 601 women, known as the CHAMACOS 1 or CHAM1 cohort, enrolled in the study, of whom 526 were followed to the delivery of a live-born, surviving singleton, of whom 263 were boys. The children were also assessed at multiple study visits approximately every 1 to 2 years, with 163 boys followed until age 12. Detailed methods for CHAM1 are published elsewhere (Eskenazi et al., 2004, 2006). When the CHAM1 children were 9 years old (2009–2011), a second cohort of 305 9-year old children, CHAMACOS 2 (CHAM2), were recruited, 161 of whom were boys, with 149 followed to age 12. Eligibility criteria were similar to those for CHAM1. Mothers gave written informed consent; children gave verbal assent starting at age 7 and written assent at age 12. All study activities were approved by the University of California at Berkeley Committee for the Protection of Human Subjects and the Centers for Disease Control (CDC) internal review board (IRB).

2.2. Data collection

CHAM1 women were interviewed twice during pregnancy (at around 13 and 26 weeks gestation), soon after delivery, and at each of the children's follow-up visits. Bilingual and bicultural interviewers conducted interviews in Spanish or English. During each interview, information was collected about family sociodemographic characteristics, maternal characteristics, and pregnancy and medical histories, as well as child developmental milestones, diet, and behavior. For CHAM2 mothers, data on pregnancy and the children's first 9 years were collected retrospectively at the child's 9-year visit. Children were interviewed privately at the 12-year old visit about their smoking and alcohol use, as well as other behaviors and habits. Children's height and weight were also measured at each of these visits, and body mass index (BMI, kg/m^2) was calculated.

Assessments of genital and pubic hair development were performed using the Tanner scale with stages from 1 (pre-puberty) to 5 (completed puberty) (Marshall and Tanner, 1970); highly trained research staff collected Tanner measurements at 9, 9.75, 10.5, 11.25, 12, and 12.75 years. Quality control of Tanner staging was overseen by a pediatric endocrinologist.

2.3. Measurement of DDT/DDE, PBDEs, and PCBs

For CHAM1 mothers, we measured the concentrations of DDT/DDE (n = 83), PBDEs (n = 82), and PCBs (n = 86) in blood serum samples collected via venipuncture during pregnancy (mean ± standard deviation = 27.4 ± 3.0 weeks gestation) or at delivery. For CHAM1 and CHAM2 participants, DDT/DDE, PBDEs, and PCBs were measured in blood serum samples from the women and their children when the children were 9 years old.

Serum samples were immediately processed and stored at −80 °C at the University of California, Berkeley, School of Public Health Biorepository until being shipped on dry ice for analysis at the CDC (Atlanta, GA). *p,p'*-DDT, *p,p'*-DDE and *o,p'*-DDT, 10 PBDE congeners (BDEs 17, 28, 47, 66, 85, 99, 100, 153, 154, and 183),

and 34 PCB congeners (International Union for Pure and Applied Chemistry numbers 18, 28, 44, 49, 52, 66, 74, 87, 99, 101, 105, 110, 118, 128, 138, 146, 149, 151, 153, 156, 157,167, 170, 172, 177, 178, 180, 183, 187, 189, 194, 195, 196,199, 206, and 209) were detected using gas chromatography isotope dilution high resolution mass spectrometry (GC-ID-HRMS). The limits of detection (LODs) for maternal samples were 0.8–6.7 ng/g lipid for DDT and DDE, 0.2–2.6 ng/g lipid for BDE-47, 0.2–0.7 ng/g for other PBDE congeners, and 0.008–2.14 ng/g lipid for PCBs. The LODs for child samples were 1.2–9.9 for DDT and DDE, 0.7–5.8 for BDE 47, 0.3–2.9 for other PBDE congeners, and 0.2–6.2 ng/g lipids for PCBs. For maternal samples taken at 9 years, the LODs were 0.6–9.75 for DDT and DDE, 0.7–5.8 for BDE-47, and 0.2–2.9 for the other PBDE congeners. Each run of 24 unknowns included quality control samples (n = 3) and method blank samples (n = 3). The analytical procedure has been published previously (Sjodin et al., 2008).

Concentrations are expressed on a serum lipid basis (nanograms/g lipids); total serum lipid concentrations were determined based on the measurement of triglycerides and total cholesterol using standard enzymatic procedures (Roche Chemicals, Indianapolis, IN), and the total lipid concentration was calculated based on an equation including the total cholesterol and triglyceride concentration (Phillips et al., 1989).

For 24 CHAM1 mothers in our final sample who lacked a prenatal blood sample but had blood collected at delivery, we estimated prenatal concentrations using predicted values from a univariate regression of delivery on prenatal concentrations. The correlations between prenatal and delivery exposure concentrations were all ≥0.95, so other predictors were not considered necessary (all regression r^2 values were ≥0.9) (Castorina et al., 2011). For both PBDEs and PCBs, the machine-read values were assigned for values less than the LOD if a signal was detected; and if no signal was detected, levels were imputed at random based on a log-normal probability distribution below the LOD via maximum likelihood estimation (Lubin et al., 2004).

For all CHAM2 children plus the 27 CHAM1 children whose mothers were missing prenatal or delivery measurements (n = 149), we predicted prenatal *p,p'*-DDT or *p,p'*-DDE concentrations using the levels in maternal and child blood taken at the 9-year visit in combination with other covariates (mothers' age, education, marital status, parity, and poverty status, duration of breastfeeding, and maternal weight and BMI both before pregnancy and when her child was 9 years old) (Verner et al., 2015). Models predicting the four primary prenatal penta-PBDE congeners (n = 142) were generated based on serum concentrations at the 9-year visit for mothers only, since inclusion of child's levels yielded a weaker predictive model (Verner et al., 2015). Prediction methods were developed using a Super Learner algorithm (Van der Laan et al., 2007), which yielded the following r^2 values in the larger sample that these predictions were based on: *p,p'*-DDT = 0.95, *p,p'*-DDE = 0.96, BDE–47 = 0.79, BDE–99 = 0.67, BDE–100 = 0.80, and BDE–153 = 0.85 (Verner et al., 2015). We did not develop a prediction model for PCBs because of the number of different congeners that would need to be considered and the low detection frequency of most.

2.4. Measurements of hormones

Concentrations of LH, FSH, and T were measured in serum samples collected before 9 AM. Of the 312 boys who completed the 12-year visit, 234 (112 boys from CHAM1 and 122 boys from CHAM2) provided a blood sample of sufficient volume for hormone analyses. (One child was missing an FSH value due to an insufficient quantity of sample.) A total of 211 of these 234 boys had measurements of DDT/DDE, PBDEs, and PCBs from blood collected at their 9-year visit.

LH (lower limit of quantification (LLOQ) = 0.017 mIU/ml) and FSH (LLOQ = 0.005 mIU/ml) were determined by electrochemiluminescent assay. Blanks, repeats, and internal standards were included for quality assurance. The intra- and inter-assay coefficients of variation (CVs) for both assays were less than 6% and 7%, respectively. T levels were analyzed using liquid chromatography with mass spectrometry detection after nonpolar solvent extraction; the LLOQ was 2.5 ng/dL. Samples were processed with inclusion of water blanks, 5 assay control pools, and a 200 pg standard to assess accuracy and precision of the assay. The intra-assay CV was <6% and the inter-assay CV was <10%. All assays were performed by Esoterix Laboratory Services (Calabasas Hills, CA).

2.5. Statistical analysis

Hormone and exposure variables all showed right-skewed distributions and were $\log_{10}$-transformed to more closely approximate a normal distribution. Thus, models are interpreted as the percent change in hormone levels per 10-fold increase in the concentration of the analyte(s). Because of the low detection for *o,p'*-DDT (26.7%), we restricted our analysis of DDT/DDE to *p,p'*-DDT and *p,p'*-DDE. We selected for statistical analysis the penta-PBDE congeners (BDE-47, −99, −100, and −153) since their detection frequencies were greater than 75%. Penta-BDE congeners were analyzed individually and as a variable summing the four congeners. Only individual PCB congeners with a detection frequency greater than 75% (PCBs 18, 28, 44, 49, 52, 66, 74, 99, 101, 118, 138, 146, 153, 156, 170, 180, 187, 194, 196, and 199 for maternal samples; and PCBs 28, 118, 138, 153, and 180 for child 9-year samples) were considered in statistical analyses. Because PCBs may have different modes of hormonal action, in maternal models we classified congeners based on dioxin-like (PCBs 118, 156, 170, 180) and non-dioxin-like (PCBs 18, 28, 44, 49, 52, 66, 74, 99, 101, 138, 146, 153, 187, 194, 196, and 199) activity (Van den Berg et al., 2006). In child serum samples, only five PCB congeners exhibited sufficient detection, and those were dominated by a single congener (PCB28); as a result, we did not divide child PCB concentrations into dioxin-like and non-dioxin-like categories.

Models with maternal DDT/DDE and PBDEs were run in two different ways: using measured concentrations only and using both measured and extrapolated maternal prenatal concentrations. We also constructed models using the measured levels of *p,p'*-DDT, *p,p'*-DDE, and PBDEs (BDE-47, 99, 100, 153, and their sum) in child serum collected when the children were 9 years of age.

Covariates that were considered for inclusion included mothers' age at menarche and pre-pregnancy BMI; maternal age, race, years in the United States, and education at time of delivery; children's birthweight, gestational age at birth, birth order, and duration of exclusive breastfeeding; children's exact age, BMI, smoking, and alcohol use status at age 12 years; and family poverty status at each visit (see Table 1). Covariates were chosen based on a directed acyclic graph (see Supplemental Figs. 1 and 2) and included maternal education and family poverty at age 9, as well as child age at hormone assessment and cohort (CHAM1 or 2) (if applicable). Models with maternal exposures also included maternal pre-pregnancy BMI. Child's age was treated as a continuous variable; family poverty, maternal education and pre-pregnancy BMI, and cohort were parameterized as categorical variables (see Table 1). Twenty-three mothers in the CHAM2 cohort were missing pre-pregnancy BMI values, so we averaged the maternal BMI values at 9, 10.5, and 12-year visits and formed categories using those values (<25, 25–30, and >30 kg/m^2). We included Tanner genital development stage in sensitivity analyses in order to determine whether *in utero* chemical exposure was related to hormone levels within a given pubertal stage. Thirteen boys were missing Tanner data at 12 years. In sensitivity analyses, we included both maternal and child mea-

Table 1
Demographics and medical characteristics of boys followed to age 12 years, CHAMACOS. 1999–2014.

Maternal Characteristics	N (%)
Age at delivery, years	
18–24	96 (41.0)
25–29	73 (31.2)
30–34	40 (17.1)
35–45	25 (10.7)
Education	
≤ 6th grade	94 (40.3)
7-12th grade	82 (35.2)
≥ High school	57 (24.5)
Race	
Latino	227 (97.4)
Other	6 (2.6)
Country of birth	
U.S.	23 (9.8)
Other	211 (90.2)
Years in the U.S.	
≤1	55 (23.5)
2–5	53 (22.7)
6–10	61 (26.1)
11+	45 (19.2)
Entire life	20 (8.6)
At or below the Census poverty line at 9 years postpartum	
No	63 (26.9)
Yes	171 (73.1)
Parity	
0	77 (32.9)
1	68 (29.1)
2+	89 (38.0)
Pre-pregnancy BMI, (kg/m^2)	
<18.5	3 (1.3)
18.5–24.9	85 (36.3)
25–29.9	83 (35.7)
≥30	63 (26.9)
Age at menarche, years	
<12	42 (18.0)
12–13	112 (47.9)
>13	80 (34.2)
Alcohol consumption during pregnancy	
No	202 (86.7)
Yes	31 (13.3)
Smoked during pregnancy	
No	230 (98.3)
Yes	4 (1.7)
Child Characteristics	N (%)
At Birth	
Year of birth	
2000	73 (31.2)
2001	102 (43.6)
2002	59 (25.2)
Low birthweight (<2500 g)	
No	219 (94)
Yes	14 (6.0)
Preterm birth (<37 weeks)	
No	214 (91.5)
Yes	20 (8.5)
Delivered by Cesarean Section	
No	188 (80.3)
Yes	46 (19.7)
Exclusively breastfed, months	
None	18 (7.9)
<2	106 (46.3)
2–4	34 (14.6)
>4	71 (31.0)
At Age 12	
BMI percentiles	
<85th percentile	95 (41.1)
85–95th percentile	48 (20.8)
>95th percentile	90 (38.6)
Tanner genital stage 2+	
No	25 (11.3)
Yes	196 (88.7)
Tanner pubic hair stage 2+	
No	111 (50)
Yes	111 (50)
Ever smoked	
No	226 (96.6)
Yes	8 (3.4)
Ever tried alcohol	
No	195 (83.3)
Yes	39 (16.7)

surements in the same model in order to assess their joint effects, although in some cases their correlation was high.

To test for a non-linear relationship between exposures and outcomes, we also ran a generalized additive model with a 3-df cubic spline for the exposure. If the model suggested evidence of non-linearity, we examined GAM residual plots and models with blood concentrations categorized by tertiles to assess the shape of the relationship. Models were also run including all 3 classes of chemicals (*p,p'*-DDT or *p,p'*- DDE, ΣPBDEs, ΣPCBs) in order to determine which class of compounds may have been driving observed associations. Possible interactions between the different classes of chemicals were examined using cross-product terms, with p-values below 0.1 considered statistically significant interactions.

In addition, in models of child and of maternal exposure, we examined mediation by the child BMI with a counterfactual approach, using indirect effects as evidence of mediation (Valeri and Vanderweele, 2013).

All models used robust standard errors based on the Huber-White sandwich estimator to account for deviation from linear regression model assumptions (Huber, 1967; White, 1980). All analyses were completed with Stata version 13.1 (Stata Corporation, College Station, Texas).

3. Results

Most of the mothers were Latina (97.4%) and born in Mexico (88.0%), with approximately half being in the United States for less than five years at the time of the index birth (Table 1). About three-quarters of the women had less than a high school education, with 40.3% having only a grade school education. The preterm birth rate in this sample was 8.5%. Almost all the boys had been breastfed (92.1%) with 31% exclusively breastfed for more than four months. At the time of the 12-year follow-up, 20.8% of the boys were overweight and 38.1% were obese, and almost all boys (88.7%) had reached Tanner genital stage 2. Few of the boys had tried smoking (3.4%) or alcohol (16.7%) at the time of their 12-year visit. Compared to boys who dropped out before age 12, those with hormone data at 12 years had older mothers, were breastfed longer, were less likely to be born by Cesarean section, and had marginally higher total maternal prenatal PCBs. No other baseline characteristics differed between those who were followed up and those who dropped out.

Table 2 shows the distribution of maternal serum concentrations for DDT/DDE, PBDEs, and PCBs. Median concentrations of *p,p'*-DDT and *p,p'*-DDE were 12.6 ng/g lipid (Interquartile Range (IQR) = 7.1, 50.3) and 496.9 ng/g lipid (IQR = 260.4, 1621.6), respectively, including measured and extrapolated values. Median concentrations of measured samples only were very similar: 12.7 ng/g lipid (IQR = 6.8, 57.2) for *p,p'*-DDT and 567.6 ng/g lipid (IQR = 214.3, 1622.2) for *p,p'*-DDE, with 100% detection. The median of the sum of the four PBDE congeners (BDE-47, −99, −100, −153) was 24.3 ng/g lipid (IQR = 15.4, 37.6), including both measured and extrapolated prenatal maternal levels; the preponderance of the sum was contributed by BDE-47. The PBDE concentrations were similar when we restricted the sample to those with measured levels (see Table 2), with nearly 100% detectable for each congener. The median of the sum of PCBs was 65.8 ng/g lipid (IQR = 43.3, 100.3) with 100% detection for at least one congener. Measured maternal serum concentrations of *p,p'*- DDT and *p,p'*-DDE were highly correlated ($r = 0.89$, $p < 0.001$), whereas these both showed a weak negative correlation with ΣPBDE ($r = -0.16$, $p = 0.14$ and $r = -0.05$, $p = 0.66$, respectively) and ΣPCB ($r = -0.14$, $p = 0.25$ and $r = -0.03$, $p = 0.82$, respectively); maternal concentrations of ΣPBDE and ΣPCB had a weak positive correlation ($r = 0.12$, $p = 0.34$).

Table 2 also shows the distribution of concentrations for DDT/DDE, PBDEs, and PCBs measured in the boys' sera at 9

Table 2
Distribution and geometric means of concentrations of biological measures of environmental chemicals in maternal and child sera, CHAMACOS.

					Percentile					
Exposure	n	% Detected	GM (GSD)	Min	10th	25th	50th	75th	90th	Max
Maternal PBDEs, ng/g lipids (measured and extrapolated)[a]										
ΣPBDEs	224		25.1 (2.2)	2.6	11.3	15.4	24.3	37.6	60.7	860.6
BDE-47	224		14.9 (2.3)	0.5	6.1	9.4	14.7	23.5	36.5	514.8
BDE-99	224		4.5 (2.3)	0.3	1.9	2.7	4.4	6.9	11.6	113.2
BDE-100	224		2.8 (2.2)	0.4	1.2	1.7	2.6	4.4	7.8	85.2
BDE-153	224		2.3 (2.2)	0.2	1.0	1.5	2.1	3.5	6.2	158.6
Maternal PBDEs, ng/g lipids (measured only)[b]										
ΣPBDEs	82	100%	23.3 (2.6)	2.6	7.6	12.5	23.4	39.4	60.7	475.7
BDE-47	82	98.8%	13.8 (2.8)	0.5	4.3	6.6	15.4	24.9	36.3	306.9
BDE-99	82	97.6%	4.0 (2.8)	0.3	1.3	2.3	3.6	7.0	12.6	113.2
BDE-100	82	96.3%	2.5 (2.5)	0.4	0.8	1.5	2.4	4.3	6.9	52.1
BDE-153	82	97.6%	2.2 (2.3)	0.2	0.9	1.3	2.2	3.4	5.8	27.6
Child PBDEs, ng/g lipids (measured only)										
ΣPBDEs	211	100%	63.9 (2.1)	7.4	25	41.4	62.8	112.5	185.4	392.4
BDE-47	211	99.5%	34.7 (2.3)	2.1	11.2	21.1	35.0	60.4	102.9	196.9
BDE-99	211	97.6%	7.8 (2.5)	0.7	2.3	4.2	7.7	14.5	28.5	76.7
BDE-100	211	99.6%	7.4 (2.2)	0.4	2.6	4.6	7.8	11.9	20.8	40.5
BDE-153	211	99.5%	10.5 (2.1)	0.5	4.3	6.5	10.0	15.1	26.9	173.1
Maternal DDT and DDE, ng/g lipids (measured and extrapolated)										
p,p'-DDT	232		24.1 (5.6)	2.6	4.9	7.1	12.6	50.3	482.2	6678.4
p,p'-DDE	232		683.8 (3.8)	34.8	181	260.4	496.9	1621.6	5336.6	34605.3
Maternal DDT and DDE, ng/g lipids (measured only)										
p,p'-DDT	83	100%	26.0 (6.7)	2.6	4.7	6.8	12.7	57.2	400.4	6678.4
p,p'-DDE	83	100%	713.1 (4.3)	73.3	146.7	214.3	567.6	1622.2	8208.7	34605.3
Child DDT and DDE, ng/g lipids (measured only)[c]										
p,p'-DDT	211	16.6%	1.8 (2.4)	0.6	0.9	1.0	1.4	2.6	5.6	194.4
p,p'-DDE	210	100%	173.0 (3.1)	21.3	51.5	79.5	137.3	295.9	898.7	12385
Maternal PCBs, ng/g lipids (measured only)										
ΣPCBs	86	100%	66.0 (1.8)	16.1	31.1	43.3	65.8	100.3	148.1	247.7
Dioxin-like	86	100%	5.0 (2.0)	0.5	2.3	3.6	5.1	7.5	11.0	32.1
Non-dioxin-like	86	100%	60.0 (1.8)	12.9	27.5	39.1	60.6	91.9	141.4	219.7
Child PCBs, ng/g lipids (measured only)										
ΣPCBs	211	100%	21.4 (2.1)	3.1	9.3	13.5	20.8	31.1	54.5	194.5

[a] Measured in prenatal (i.e., from pregnancy or from delivery) serum or back- extrapolated from 9-year visit.
[b] Measured in prenatal serum.
[c] Measured in boys' sera at 9- year visit.

years of age. The median level of *p,p'*-DDT in children was low (median = 1.4 ng/g lipid; IQR = 1.0, 2.6) compared to mothers' (median = 12.7 ng/g lipid, IQR = 6.8, 57.2), and only 16.6% of samples contained detectable levels. The median level of child *p,p'*-DDE (median = 137.3 ng/g lipid; IQR = 79.5, 295.9) was less than a quarter of measured maternal levels (median = 567.6 ng/g lipid; IQR = 214.3, 1622.2), but with 100% detection. The median of the child's ΣPCB (median = 20.8 ng/g lipid; IQR = 13.5, 31.1) was less than a third of the mothers' levels (median = 65.8 ng/g lipid; IQR = 43.3, 100.3). In contrast, the median of the ΣPBDE congeners was more than twice as high in child than in maternal serum (median = 62.8 ng/g lipid; IQR = 41.4, 112.5). Correlations between measured maternal prenatal and child 9-year *p,p'*-DDT, *p,p'*-DDE, ΣPBDE, and ΣPCB levels were 0.81 ($p < 0.001$), 0.76 ($p < 0.001$), 0.18 ($p < 0.13$), and 0.18 ($p < 0.14$), respectively.

Table 3 shows the serum levels of FSH, LH, and testosterone in the CHAMACOS boys at 12 years old. The median concentration of each hormone was as follows: FSH was 2.5 mIU/mL serum (IQR = 1.8, 3.4), LH was 2.3 mIU (IQR = 1.3, 3.7), and T was 74.5 ng/dL (IQR = 18, 272).

Table 4 presents the adjusted models for DDT/DDE, PBDEs, and PCBs in maternal prenatal serum and hormone levels in the 12-year old boys. Maternal serum *p,p'*-DDT (measured only, or measured and extrapolated) were somewhat associated with decreases in the boys' LH and T, which became statistically significant when we adjusted for Tanner stage (see Table 4, Supplemental Table 1). Specifically, after controlling for Tanner stage, a 10-fold increase in maternal *p,p'*-DDT concentrations was associated with an 18.5% decrease in LH (95% confidence interval (CI) = −29.8, −5.4) and an 18.2% decrease in T (95% C = −30.2, −4.2) (See Supplemental Table 1). Similarly, a 10 -fold increase in *p,p'*-DDE was related to an 18.3% decrease in LH (95% CI = −32.9, −0.6), when we controlled for Tanner stage.

In contrast, prenatal maternal serum concentrations of ΣPBDE (measured and extrapolated) were associated with an increase in the boys' LH levels (adjusted percent change per 10-fold increase = 51.0%; 95% CI = 3.0, 121.3) but with no relationship with FSH or T (Table 4). For individual congeners, maternal concentrations of BDE-100 were associated with an increase in LH levels (adjusted percent change per 10-fold increase = 75.1%; 95% CI = 21.8, 151.6), while maternal BDE-153 concentrations were associated with increases in FSH (adjusted percent change per 10-fold increase = 22.2%; 95% CI = 1.0, 47.9), LH (adjusted percent change per 10-fold increase = 96.6%; 95% CI = 35.7, 184.7) and T (adjusted percent change per 10-fold increase = 92.4%; 95% CI = 20.9, 206.2). When analysis was restricted to measured maternal levels only, results were similar except that maternal concentrations of BDE-100 were now also associated with an increase in boys' T levels (adjusted percent change per 10-fold increase = 118.7%; 95% CI = 10.6, 332.2), and maternal BDE-153 concentrations were no longer associated with the boys' FSH levels. Supplemental Table 1 shows that even after controlling for the boys' Tanner stage, significant positive associations were found between BDE-100 (measured and extrapolated) and LH (adjusted percent change per 10-fold increase = 58.9; 95% CI = 5.0, 140.4) and between BDE-153 and LH (adjusted percent change per 10- fold increase = 79.0; 95% CI = 19.2, 168.8) and T (adjusted percent change per 10-fold increase = 56.0; 95% CI = 16.9, 108.2).

Similarly, maternal prenatal ΣPCBs levels had positive relationships with hormone levels of the 12-year olds (Table 4) but were

Table 3
Hormone levels (FSH, LH, and testosterone) in boys at age 12, CHAMACOS cohort, 2012–2014.

				Percentile					
Outcome	N	GM (GSD)	Min	10th	25th	50th	75th	90th	Max
FSH	233	2.0 (2.0)	0.16	1.3	1.8	2.5	3.4	4.7	9.5
LH	234	2.0 (3.0)	0.01	0.53	1.3	2.3	3.7	5.1	13.0
Testosterone	234	69.0 (4.0)	4.3	9.4	18.0	74.5	272	422	780

FSH and LH concentrations are in mIU/mL serum. Testosterone concentrations are in ng/dL serum.

Table 4
Adjusted linear regression β coefficient and 95% confidence interval (CI) for the associations between prenatal DDT/DDE, PBDE, and PCB exposure and the percent change in hormone levels of 12-year old boys, CHAMACOS.

Exposure	N	FSH (% change & CI)	LH (% change & CI)	Testosterone (% change & CI)
Maternal DDT and DDE (measured and extrapolated)				
pp'-DDT	232	−5.0 (−13.0, 3.7)	−12.9 (−26.0, 2.4)	−18.0 (−34.9, 3.3)
pp'-DDE	232	−9.2 (−18.9, 1.6)	−12.9 (−29.6, 7.7)	−21.1 (−42.2, 7.6)
Maternal DDT and DDE (measured only)				
pp'-DDT	83	7.7 (−6.0, 23.5)	−9.6 (−28.7, 14.5)	−10.3 (−36.0, 25.7)
pp'-DDE	83	6.3 (−11.1, 27.2)	−6.8 (−34.1, 31.6)	−7.2 (−42.4, 49.3)
Maternal PBDEs (measured and extrapolated)				
ΣPBDEs	224	12.4 (−7.5, 36.5)	51.0 (3.0, 121.3)*	36.2 (−21.5, 136.3)
BDE-47	224	8.6 (−9.6, 30.6)	37.4 (−5.7, 100.2)	18.5 (−32.0, 106.7)
BDE-99	224	9.8 (−9.9, 33.8)	36.9 (−4.5, 96.0)	31.1 (−22.8, 122.5)
BDE-100	224	14.9 (−5.7, 40.0)	75.1 (21.8, 151.6)*	55.6 (−6.0, 157.6)
BDE-153	224	22.2 (1.0, 47.9)*	96.6 (35.7, 184.7)*	92.4 (20.9, 206.2)*
Maternal PBDEs (measured only)				
ΣPBDEs	82	−0.1 (−24.0, 31.4)	59.8 (−11.6, 188.9)	94.2 (−7.6, 308.1)
BDE-47	82	−0.3 (−22.4, 28.2)	44.7 (−18.3, 156.3)	66.9 (−20.2, 248.8)
BDE-99	82	1.6 (−21.9, 32.1)	48.4 (−9.6, 143.5)	72.9 (−10.3, 233.3)
BDE-100	82	−5.8 (−28.9, 24.9)	84.0 (8.7, 211.5)*	118.7 (10.6, 332.2)*
BDE-153	82	0.2 (−26.0, 35.7)	140.3 (40.1, 312.1)*	207.1 (53.8, 513.3)*
Maternal PCBs (measured only)				
ΣPCBs	86	64.5 (8.6, 149.0)*	42.0 (−45.1, 267.3)	153.9 (−30.5, 827.9)
Dioxin-like	86	9.1 (−18.8, 46.7)*	31.7 (−32.0, 155.2)	84.6 (−28.7, 377.7)
Non-dioxin-like	86	63.3 (9.3, 143.9)*	33.7 (−46.5, 234.0)	124.2 (−37.3, 702.0)

All models include maternal education and pre-pregnancy BMI, family poverty status at 9 years, child age, and cohort (where applicable). All exposures and outcomes are log10- transformed; therefore, the results reported represent the percent change in hormones per 10-fold increase in chemical concentration (data not shown).
* $p < 0.05$.

only significantly associated with FSH levels. Specifically, maternal prenatal ΣPCB concentrations were related to a 64.5% increase in boys' FSH levels (95% CI = 8.6, 149.0). This relationship is driven by the non-dioxin-like PCBs (adjusted percent change per 10-fold increase = 63.3%; 95% CI = 9.3, 143.9. The results controlling for Tanner stage were similar.

Although numbers were appreciably smaller (n = 69 or 70), when we included the measured concentrations of ΣPBDE, ΣPCB and *p,p'*-DDT or *p,p'*-DDE in the same model, only ΣPCB remained associated with elevated FSH (see Supplemental Table 2). We found no statistical interaction between different exposures when added to the above models.

There was no evidence of non-linearity in the relationships presented above and no evidence of mediation of child BMI on the association of maternal chemical concentrations and child hormone levels (data not shown).

As shown in Table 5, child BDE-153 levels at age 9 were associated with an increase in LH (adjusted percent change per 10-fold increase = 59.0% 95% CI = 8.6, 132.6) and T (adjusted percent change per 10-fold increase = 149.1%; 95% CI = 42.7, 334.6) and child ΣPCB concentrations with an increase in T at age 12 years (adjusted percent change per 10-fold increase = 108.9%; 95% CI = 7.1, 307.2). However, these associations appear to be primarily mediated by the child's BMI with no evidence of a direct effect of chemical concentrations on hormones. Specifically, child BMI mediated 42.0% of the association of child BDE-153 levels and LH, 30.3%, of BDE-153 and T, and 47.7% of ΣPCB and T. No other associations were found between the child's chemical concentrations and hormone levels. Adding Tanner stage to these models did not appreciably change the results (see Supplemental Table 3). We found no statistical interaction between different exposures when added to the above models, and no evidence of non- linearity in the relationships presented above.

When both maternal and child chemical concentrations were included in the same model, the results are similar to those found in the above separate models.

4. Discussion

We examined the relationship of maternal prenatal DDT, DDE, PBDEs, and PCBs serum concentrations with the sex hormone levels of their sons at 12 years of age. We found that mothers' levels of DDT/DDE were somewhat inversely related to their sons' sex hormones in our primary models, and *p,p'*-DDE became significantly inversely associated with LH, and *p,p'*- DDT with LH and T, when Tanner stage was considered in the model. We also found that ΣPBDE concentrations, specifically of BDE-100 and −153, were associated with increased hormone levels (BDE-100 with LH and BDE- 153 with FSH, LH and T). Maternal ΣPCB concentrations, specifically the non-dioxin-like PCBs, were associated with increased FSH. Any associations found between the chemical concentrations in the children's blood at 9 years and their hormone levels at 12 years appeared to be mediated by the child's BMI.

Although cross-sectional studies of primarily European or US adults (Cocco et al., 2004; Ferguson et al., 2012; Goncharov et al., 2009; Hagmar et al., 2001) have reported no association between DDT or DDE and sex hormones, serum *p,p'*-DDE has been associated with lowered T in more highly-exposed populations such as Mexi-

Table 5
Adjusted linear regression β coefficient and 95% confidence interval (CI) for the associations between DDT/DDE, PBDE and PCB serum concentration in the boys' at 9 years and the percent change in hormone levels at 12 years in the CHAMACOS cohort.

Exposure	N	FSH (% change & CI)	LH (% change & CI)	Testosterone (% change & CI)
p,p'- DDT	211	1.7 (−13.9, 20.1)	−12.8 (−36.3, 19.5)	−16.6 (−47.1, 31.5)
p,p'- DDE	210	−4.3 (−18.1, 11.8)	5.8 (−24.9, 49.0)	7.5 (−29.6, 64.1)
ΣPBDEs	211	0.3 (−21.2, 27.6)	21.6 (−29.1, 108.4)	40.4 (−26.2, 167.2)
BDE-47	211	0.9 (−18.9, 25.5)	14.3 (−31.5, 90.8)	19.6 (−33.5, 115.1)
BDE-99	211	−0.7 (−17.9, 20.1)	9.1 (−30.6, 71.7)	4.3 (−37.5, 74.3)
BDE-100	211	0.1 (−19.4, 24.5)	13.0 (−31.2, 85.6)	18.1 (−37.1, 121.6)
BDE-153	211	8.0 (−10.7, 30.6)	59.0 (8.6, 132.6)[*, †]	149.1 (42.7, 334.6)[*, †]
ΣPCBs	211	8.8 (−14.1, 37.8)	26.8 (−28.0, 123.3)	108.9 (7.1, 307.2)[*, †]

All models include maternal education, poverty status at 9 years, child age, BMI, smoking & alcohol use at 12 years of age, gestational age at birth, and cohort. All exposures and outcomes are log10-ransformed; therefore, the results reported represent the percent change in hormones per 10-fold increase in chemical concentration.

[*] $p < 0.05$.

[†] Association mediated by child BMI: 42.0% of total association between LH and BDE-153, 30.3% of association of testosterone and BDE- 153, and 47.7% of association between testosterone and ΣPCBs.

can flower growers (Blanco-Munoz et al., 2012), residents of a rural Brazilian village where a chemical plant had been located (Freire et al., 2014), and a small sample of highly-exposed 16–28 year old Mexican men (Ayotte et al., 2001). In addition, Martin et al. found a decrease in T levels and free androgen index in African-American farmers, but only in those in the highest 10th percentile of *p,p'*-DDE levels (2002). In contrast to the above findings, a relatively small study of South-African malaria vector- control workers (n = 47) who were highly-exposed (Dalvie et al., 2004) reported significant positive relationships between *p,p'*-DDT and both estradiol and T, but not with FSH or LH; they reported no significant relationships with *p,p'*-DDE. The only previous study examining the relationship of maternal prenatal exposure to DDT/DDE and adolescent hormones reported null findings (Gladen et al., 2004). Similar to some of the cross-sectional studies of men (Ayotte et al., 2001; Blanco-Munoz et al., 2012; Freire et al., 2014; Martin et al., 2002), we find some evidence of an inverse association of DDT/DDE and male hormones in the adolescents that are only statistically significant when we controlled for Tanner stage (see Supplemental Table 1), suggesting that within a given stage of puberty there may an inverse relationship with hormone levels. Diminished secretion of T can result in failure of gonadal function (hypogonadism), such that males fail to produce normal numbers of sperm (Dandona and Rosenberg, 2010). Thus, if the observed relationship continues into adulthood, a reduction in fertility may be noted.

We report, in this first study of *in utero* PBDE exposure and male adolescent hormones, that maternal blood concentrations of the longer half-life congeners BDE-100 and −153 were associated with increases in some of the male hormones. Increased male hormones could result in earlier onset of puberty in these boys (possibly mediated via alteration of the hypothalamic–pituitary–gonadal axis). LH (which acts on Leydig cells in the testes to produce T) and FSH (which acts on Sertoli cells in the testes to initiate spermatogenesis) work synergistically and are generally elevated in male precocious puberty (Blondell et al., 1999; Dandona and Rosenberg 2010). However, there are few previous studies in humans to support our findings and results in animals are conflicting. For example, in animal studies, prenatal administration of BDE-99 at environmentally-relevant doses resulted in adult male offspring with smaller tested and epididymus, and lower sperm production, but no effect on LH or T (Kuriyama et al., 2005), whereas another study found decreased estrogen and T and nonsignificantly earlier onset of puberty (Lilienthal et al., 2006), and yet another study found that PBDE-710 (a mixture of tetra- and penta-BDE) stimulated T secretion in rat Leydig cells (Wang et al., 2011). A previous cross-sectional study of men found inverse or no association with the same male hormones examined in the present study (Johnson et al., 2013; Meeker et al., 2009).

PCBs are mixtures of congeners with varying modes of action (Wolff et al., 1997); some act like dioxins on the aryl hydrocarbon receptor, and others are non-dioxin-like compounds, acting primarily as neurotoxicants by affecting the hypothalamic–pituitary–gonadal axis (Dandona and Rosenberg, 2010). Our finding that maternal prenatal serum concentrations of PCBs were positively associated with FSH in adolescents, particularly of non-dioxin-like congeners, shows mixed consistency with results of previous studies, possibly due to varying mixes of PCB congeners. PCB cord levels showed an inverse association with T and LH in 14-year old boys from the Faroe Islands birth cohort study (Grandjean et al., 2012). Their exposure was predominantly from consumption of whale blubber and to non-dioxin-like PCBs, for which we found positive associations with sex hormones. The nonsignificant findings in the Yucheng cohort, in which adolescents were exposed primarily to dioxin-like PCBs and dibenzofurans following a rice oil contamination, are more in line with the lack of association we found between dioxin-like PCBs and adolescent male reproductive hormones (Hsu et al., 2005). The overall levels of exposure in both the Faroe Islands and Yucheng studies are likely considerably higher than in our population, which had lower levels than the general US population participating in NHANES (CDC, 2005). The similarity in our findings from *in utero* exposure to PBDEs and to PCBs is noteworthy, given their resemblance in structure (Siddiqi et al., 2003).

While we found associations between male hormones in the 12-year olds and childhood (at 9-years) concentrations of BDE-153 (LH and T) and total PCBs (T only), these associations appear to be mediated by child BMI. We previously reported a positive relationship with body mass in these CHAMACOS boys at 7-years of age of maternal prenatal BDE-153 concentrations, but an inverse relationship of child BDE-153 concentrations (Erkin-Cakmak et al., 2015). Taken together, our results suggest a complex interrelationship between childhood PBDE levels, especially for the more lipophilic compounds, and body mass, pubertal onset, and male sex hormones (Chevrier, 2013).

The strengths of the present study are its unique longitudinal design, the use of prenatal as well as child blood chemical concentrations with a wide range of exposure, and extensive covariate information for both mothers and children. In addition, the population is relatively homogenous with regard to both race and social class, which potentially reduces the influence of unmeasured confounding. Furthermore, all boys were 12 years old, and we have considered Tanner staging in the analysis. However, the study is limited in that we use back-extrapolated exposure measures to estimate *in utero* exposure to DDT/DDE and PBDEs for some of the cohort, although results were similar in most cases when restricting the analysis to the group with measured levels. *In utero* concentrations of PBDEs and PCBs were similar or lower that the general US

population's; because the maternal DDT/DDE levels were relatively high due to most mothers' recent immigration from Mexico, results on DDT/DDE may not be generalizable to the general US population. In addition, we did not measure some hydroxy-BDEs that may be more biologically active (Siddiqi et al., 2003), nor the highly-brominated compounds of PBDEs such as BDE-209, although they likely represent a small portion of the total PBDE body burden (Rose et al., 2010).

In conclusion, in this prospective follow-up study, we found that maternal prenatal blood concentrations of PCBs, PBDEs and DDT/DDE were associated with some alterations in serum hormone concentrations in adolescent boys, particularly for the higher congener PBDEs and the non-dioxin-like PCBs. Any associations of childhood blood concentrations of these endocrine-disrupting chemicals and sex hormones are likely mediated by associations of exposure with child BMI. Thus, these results suggest endocrine disruption due to exposures that occurred prenatally. Although the implications on future testicular and reproductive function in puberty and in adulthood are not known at this time, we are currently evaluating the relationship with *in utero* exposure to these endocrine disruptors and timing and tempo of puberty. Given the paucity of studies examining *in utero* exposures and male reproductive hormones and the inconsistencies in findings across the few existing studies, more research is needed to confirm our results and to determine whether the purported effects of these endocrine disruptors persist into adulthood, when hormone levels may be more stable.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijheh.2016.11.001.

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