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Short running title: Organochlorine pesticides and male sexual maturity

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Abstract

Background: Few human studies have evaluated the impact of childhood exposure to organochlorine pesticides (OCP) on pubertal development.

Objective: To evaluate associations of serum OCP concentrations [hexachlorobenzene (HCB), β-hexachlorocyclohexane (β-HCH), and *p,p*-dichlorodiphenyldichloroethylene (*p,p*'-DDE)] with age at attainment of sexual maturity among boys.

Methods: From 2003-2005, 350 8-9 year-old boys from Chapaevsk, Russia with measured OCPs were enrolled and followed annually for eight years. We used multivariable interval-censored models to evaluate associations of OCPs (quartiles) with three physician-assessed measures of sexual maturity: Tanner stage 5 for genitalia growth, Tanner stage 5 for pubic hair growth, or testicular volume (TV)≥20 mL in either testis.

Results: In adjusted models, boys with higher HCB concentrations achieved sexual maturity reflected by TV≥20 mL a mean of 3.1 months (95% CI: -1.7, 7.8), 5.3 months (95% CI: 0.6, 10.1), and 5.0 months (95% CI: 0.2, 9.8) later for quartiles Q2, Q3, and Q4, respectively compared to Q1 (trend p=0.04). Tanner stage 5 for genitalia growth was attained a mean of 2.2 months (95% CI: -3.1, 7.5), 5.7 months (95% CI: 0.4, 11.0), and 3.7 months (-1.7, 9.1) later for quartiles Q2, Q3, and Q4 respectively of β-HCH as compared to Q1 (trend p=0.09). Tanner stage 5 for pubic hair growth occurred 6-9 months later on average for boys in the highest vs. lowest quartile for HCB (trend p<0.001), β-HCH (trend p=0.01), and *p,p*'-DDE (trend p=0.04). No associations were observed between *p,p*'-DDE and Tanner stage 5 for genitalia growth or TV≥20 mL.

Conclusions and relevance: Higher prepubertal serum HCB and β-HCH concentrations were associated with a later age at attainment of sexual maturity. Only the highest quartile of serum *p,p*'-DDE was associated with later pubic hair maturation.

Introduction

Organochlorine pesticides (OCPs) such as hexachlorobenzene (HCB), β-hexachlorocyclohexane (β-HCH), and 1,1,1,-trichloro-2,2,bis(p-chlorophenyl)ethane (DDT) were used as insecticides and fungicides for decades until the 1980s (Barber et al. 2005; Jaga and Dharmani 2003; Jung et al. 1997). Though production of these pesticides has been banned in most countries (Barber et al. 2005; Breivick et al. 1999; Jaga and Dharmani 2003), DDT is still used to control for malaria (Jaga and Dharmani 2003), and HCB and β-HCH are unintentional by-products from manufacturing of other chlorinated chemicals (Courtney 1979; Jung et al. 1997). The lipophilic and persistent nature of these environmentally stable compounds and their ability to biomagnify through the food chain (Barber et al. 2005; Jaga and Dharmani 2003; Jung et al. 1997) are primary reasons for ongoing exposure in the general population. These organochlorine pesticides (OCPs) and their metabolites such as *p,p*'-DDE are endocrine disrupting chemicals (EDCs) that affect puberty and reproductive development in rodents (Courtney 1979; Gray et al. 2001; Kelce et al. 1995; Van Velsen et al. 1986).

Puberty is a complex process characterized by physical and hormonal changes regulated by two parallel but independent processes: adrenal maturation (adrenarche) and the maturation of the hypothalamic pituitary gonadal (HPG) axis (Havelock et al. 2004; Kronenberg et al. 2008). In boys, virilization of the genitalia and testicular enlargement are cues of HPG activation, while pubic hair growth is often associated with adrenarche (Havelock et al. 2004). Early attainment of male sexual maturity is associated with a variety of adverse effects including antisocial behaviors, short adult height, reduced fertility, and prostate and testicular cancer (Golub et al. 2008; Patton and Viner 2007). Later male maturity has been linked to poor body image,

depression, and osteoporosis (Golub et al. 2008; Patton and Viner 2007; Rosen and Foster 2001). Factors that may affect the timing of sexual maturation include deficits in energy/micronutrients and possibly environmental chemicals such as OCPs (Golub et al. 2008; Kaplowitz 2008; Mouritsen et al. 2010; Mustanski et al. 2004; Rogol et al. 2000).

While rodent studies have demonstrated that fetal and postnatal exposure to *p,p*'-DDE, DDT's primary metabolite, delayed male preputial separation (Kelce et al. 1995), a marker of male puberty, none have reported on associations with sexual maturation. Sexual maturity in rodents is assessed by sperm production or mating behavior. The age at production of mature sperm varies widely among strains, with estimates ranging from post-natal day 40 to 100, therefore assessing the timing of sexual maturation in rodents is imprecise (Campion et al. 2013; Marty et al. 2003). HCB, β-HCH, and *p,p*'-DDE have been associated with reproductive and developmental abnormalities in male rodent offspring including fetal growth retardation, delayed testicular descent, and reduced fertility (Courtney 1979; Gray et al. 2001; Kelce et al. 1995; Quinn et al. 2008; Simon et al. 1979; Van Velsen et al. 1986). Epidemiologic evidence on the association of OCPs with pubertal development is limited and the direction of the findings is inconsistent (Den Hond et al. 2010). In a cross-sectional study of Flemish boys aged 14-15 years, higher serum levels of HCB and *p,p*'-DDE were associated with earlier genital and pubic hair development (Den Hond et al. 2010). In contrast, among a cohort of Russian boys residing in an environmentally contaminated town, we reported that higher serum HCB concentrations were associated with later pubertal onset (Lam et al. 2014). In the present analysis, we investigated the association of prepubertal serum concentrations of HCB, β-HCH, and *p,p*'-DDE with age at male sexual maturity in the same cohort of Russian boys.

Methods

Study Population. The Russian Children's Study is a prospective cohort study of 499 boys, enrolled at age 8-9 years in 2003-2005, residing in Chapaevsk, Russia, a community contaminated with organochlorine compounds, including OCPs (Burns et al. 2009; Lam et al. 2013). Exclusion criteria included severe chronic medical conditions or institutionalization. OCP concentrations were not measured for the first 144 boys enrolled as the study initially focused on dioxins, and 5 additional boys were excluded due to chronic illnesses impacting growth, leaving 350 boys for the present analysis. The study was approved by the Human Studies Institutional Review Boards of the Chapaevsk Medical Association, Harvard School of Public Health, University of Massachusetts Medical School, and Brigham and Women's Hospital. The parent/guardian gave informed consent and the boys signed assent forms prior to participation.

At study entry, the parent/guardian completed nurse-administered health and lifestyle questionnaires on demographics, medical and family history, household smoking, breastfeeding of the child in the study, household income, and parental education. At the same visit, the parent/guardian completed a Russian Institute of Nutrition food frequency questionnaire to ascertain the boy's usual dietary intake. Birth outcomes (e.g., birth weight and gestational age) were abstracted from the medical records. Blood lead levels (BLLs) were measured from the boys' blood samples collected at enrollment (ages 8-9 years) (Hauser et al. 2008; Williams et al. 2010).

Physical Examination and Pubertal Assessment. A standardized physical examination was performed at study entry and annually for up to eight follow-up visits by a single physician

(OS). Testicular volume (TV) was measured by palpation and comparison to a Prader orchidometer. Pubertal assessments were also performed by visual inspection using established Tanner stage criteria for genitalia and pubic hair on a scale of 1 (immature) to 5 (mature) (Tanner and Whitehouse 1976). Sexual maturity was defined as Tanner stage 5 for genitalia growth, or Tanner stage 5 for pubic hair growth, or TV≥20 mL for either testis (Basu 2011).

Organochlorine Pesticide Exposure Assessment. At study entry, fasting blood samples were collected from participants, and serum aliquots stored at -35°C until shipment on dry ice to the U.S. Centers for Disease Control and Prevention, Atlanta, GA for analysis. The samples, including method blanks and quality control samples, were spiked with $^{13}C_{12}$-labeled pesticides, extracted by a C18 solid-phase extraction (SPE) followed by a multicolumn automated cleanup and enrichment procedure (Sjodin et al. 2004; Turner et al. 1997). Samples were analyzed with high-resolution mass spectrometry in selective ion monitoring mode (Barr et al. 2003). Total serum lipid content of the aliquot was determined from enzymatic measurements of total cholesterol and triglycerides (Phillips et al. 1989). The analytical coefficients of variation for individual OC pesticides in QA/QC samples ranged between 10-15%. All OCP concentrations were expressed on a wet-weight basis (pg/g serum) or on a lipid-normalized basis (ng/g lipid) (division of wet-weight levels by lipid concentrations).

Statistical Analysis. Unadjusted and adjusted interval-censored survival analyses were used to evaluate the associations between boys' serum OCP concentrations (in quartiles) and age at sexual maturity; the three higher quartiles were each compared to the lowest quartile and tests for trend were performed by modeling OCP quartiles as an ordinal variable. A normal

distribution for age at sexual maturity was assumed. Use of an interval-censored model allows for the fact that sexual maturity may occur in the interval between study visits (interval-censored), or may not yet have occurred by the last study visit (right-censored). We calculated the overall mean age of sexual maturity for each maturity measure, and the mean age of maturity for each OCP quartile assuming the mean or reference levels for other model covariates.

Covariates considered in the models included *a priori* identified potential predictors of sexual maturity at baseline (Table 1): maternal age at son's birth, household tobacco use, boys' birth weight and gestational age, breastfeeding duration, diet, and BLLs at study enrollment, as well as socioeconomic status (SES) indicators (e.g., biological father's absence from the household, household income, parental education). A core model was developed first by evaluating the associations of each covariate with sexual maturity and retaining those with a $p<0.20$. Covariates meeting this criterion were then included in a full model and backwards selection (likelihood ratio test) was used to exclude covariates with $p>0.10$. To check for confounding, covariates were added individually back into the final model and retained if they resulted in a ≥ 10% change in the OCP coefficient estimates obtained from the trend test. Separate core models were developed for each maturity measure. Since OCPs are lipophilic and due to the potential for bias, rather than modeling lipid-normalized OCPs, we instead chose to use the wet-weights for OCPs and adjust for concurrently measured serum total lipids by including it as a covariate in the model (Li et al. 2013; Schisterman et al. 2005). However, we also performed an alternative analysis using quartiles of lipid-normalized serum OCP concentrations rather than wet-weight serum OCPs. Statistical significance was defined as

$p \leq 0.05$. All statistical analyses were conducted using SAS statistical software, version 9.2 (SAS Institute Inc., Cary, North Carolina).

Prior analyses in this cohort have found OCPs to be associated with reduced BMI and height z-scores (defined according to WHO child growth standards) (Burns et al. 2012; de Onis et al. 2007): these markers of growth are, in turn, strongly associated with age at sexual maturity and thus may be on the causal pathway between OCPs and sexual maturity. Due to these previously identified relationships, BMI and height z-scores were excluded from the primary analysis, but sensitivity analyses were conducted to evaluate these mediators by adding them to the final models. Sensitivity analyses were also conducted to assess robustness of findings with further adjustment for maternal age at menarche (unavailable for 8% of participants).

Results

Exposure and demographic characteristics. Median (25^{th}, 75^{th} %-iles) concentrations for wet-weight serum HCB, β-HCH, and *p,p*'-DDE were 754 (522, 1159), 814 (560, 1294), and 1408 (904, 2324) pg/g serum, respectively. The median (25^{th}, 75^{th} %-iles) concentrations for lipid-normalized serum HCB, β-HCH, and *p,p*'-DDE were 159 (107, 247), 68 (114, 272), and 287 (189, 492) ng/g lipid, respectively. No values were below the limit of detection. Most boys had BMI and height z-scores within one standard deviation of the WHO mean (Table 1). Boys with and without serum OCP measurements (n=350 vs. 144) did not differ significantly by BMI, height z-scores, or birth characteristics at the 0.05 significance level (Lam et al. 2013). However, more boys with OCP measurements were in the highest parental education categories and household income categories than those without. Spearman correlations between the OCPs were

r=0.34 for HCB and *p,p*'-DDE, r=0.54 for β-HCH and HCB, and r=0.61 for β-HCH, and *p,p*'-DDE.

Sexual maturity characteristics. Among the 72% of boys who were followed until at least the 16-17 year old study visit, 94%, 87% and 44% had attained TV≥20 mL, Tanner stage 5 for genitalia growth, and Tanner stage 5 for pubic hair growth, respectively. The overall estimated mean age (95% confidence interval (CI)) of sexual maturity for TV≥20 mL, Tanner stage 5 for genitalia growth, and Tanner stage 5 for pubic hair growth was 13.8 (13.7, 14.0), 14.7 (14.6, 14.9), and 16.0 years (15.8, 16.2), respectively.

Associations of serum OCPs with TV≥20 mL and Tanner stage 5 for genitalia growth. The multivariable model for TV≥20 mL included the covariates total serum lipids, biological father's absence from the household, boy's birth weight, and boy's BLLs ≥ 5μg/dL. For Tanner stage 5 for genitalia growth, the multivariable model included the covariates total serum lipids and macronutrients (i.e., total caloric intake, percent calories from carbohydrates, fat and protein). Higher serum HCB concentrations were associated with later attainment of both TV≥20 mL and Tanner stage 5 for genitalia. HCB quartiles 3 and 4 were associated with approximately 5 months later TV≥20 mL compared to the lowest quartile, with a significant trend. However, only quartile 3 of HCB was associated with later attainment for Tanner stage 5 for genitalia (by 5.6 months, 95% CI: 0.3, 10.9), with attenuation in quartile 4 and no significant trend. A similar pattern was observed for β-HCH and Tanner stage 5 for genitalia growth, with a significantly later age at maturity only observed within the third quartile; no association was observed between β-HCH and reaching TV≥20 mL (Table 2, Figure 1). The estimated mean ages

at maturity ranged from 13.4-13.8 years for TV≥20 mL and from 14.5-14.8 years for attaining Tanner stage 5 for genitalia. We observed no statistically significant association for *p,p*'-DDE with later TV or genital maturity.

Associations of serum OCPs with Tanner stage 5 for pubic hair growth. The models for age at attainment of Tanner stage 5 for pubic hair growth were adjusted for total serum lipids and biological father's absence from the household. In adjusted models, boys with higher HCB and β-HCH concentrations (Q3 and Q4) attained Tanner stage 5 for pubic hair growth more than six months later, on average, than those in the lowest quartile, although the associations were attenuated in the highest quartile (HCB trend $p<0.001$; β-HCH trend $p=0.01$; Table 2, Figure 1). Adjusted mean ages at attainment of Tanner stage 5 for pubic hair growth ranged from 15.2-16.1 years over HCB quartiles and 15.4-15.9 years over β-HCH quartiles. The association of *p,p*'-DDE with later Tanner stage 5 for pubic hair growth (trend $p=0.04$) was primarily driven by quartile 4 (Figure 1).

Sensitivity analyses. In sensitivity analyses adjusted for maternal age at menarche, the associations of TV≥20 mL and Tanner stage 5 for genitalia growth with HCB and β-HCH were consistent with primary models (see Supplemental Material, Table S1). Adjustment of primary models for baseline BMI and height z-scores resulted in slight attenuation of the associations of HCB and β-HCH with TV≥20 mL and Tanner stage 5 for genitalia growth. In contrast, after adjustment for BMI and height z-scores, β-HCH and *p,p*'-DDE were no longer associated with Tanner stage 5 for pubic hair growth, whereas the association of HCB with Tanner stage 5 for pubic hair growth was attenuated but remained significant.

Associations of lipid-normalized serum OCPs with sexual maturity. Analyses modeling lipid-normalized serum OCPs yielded stronger associations of β-HCH with Tanner stage 5 for pubic hair growth, TV≥20 mL, and Tanner stage 5 for genitalia growth, compared to wet-weight models adjusted for serum lipids, but primary conclusions were unaffected (see Supplemental Material, Table S2 and Figure S1). Lipid-normalized serum HCB associations with sexual maturity were attenuated compared to the wet-weight models; the association with TV≥20 mL became non-significant. Models using lipid-normalized *p,p*'-DDE demonstrated a stronger association in comparison to wet-weight models for genitalia and TV≥20 mL, although the association with pubic hair was attenuated. Additional analyses with lipid-normalized OCP measures further adjusted for maternal age at menarche and BMI and height z-scores did not substantially change our results (see Supplemental Material, Table S3).

Associations of OCP mixtures with sexual maturity. Estimated associations of age at maturity with either HCB or β-HCH were very similar after additional adjustment for *p,p'-DDE*. However, in models including both HCB and β-HCH, associations for HCB were attenuated and remained significant only for Tanner stage 5 for pubic hair growth; associations for β-HCH, were markedly attenuated for all maturity markers and none approached significance. There were no associations of *p,p*'-DDE with Tanner stage 5 for pubic hair growth in models of multiple OCPs (Supplemental Material, Table S4).

Discussion

In our longitudinal study, we found associations of higher prepubertal serum HCB, β-HCH, and *p,p*'-DDE concentrations with later sexual maturity defined as Tanner stage 5 for

pubic hair growth, as well as an association of higher HCB with later attainment of TV≥20 mL. Our recent analysis of this Russian cohort found later pubertal onset among boys with higher serum HCB concentrations (Lam et al. 2014). These pubertal onset findings, along with the results of our current analysis on the association of HCB with later sexual maturity, suggests that there is, on average, a similar five month delay in both pubertal onset and attainment of sexual maturation. Thus, on average, the pace (tempo between onset and sexual maturity) of puberty did not change in relation to HCB exposure.

Few epidemiologic studies have assessed the association of OCPs with age at sexual maturity and the findings have been inconsistent, possibly due to differences in study design, definition of maturity, exposure mixtures, and timing of exposure and outcome assessment (Den Hond et al. 2010; Gladen et al. 2000). A prospective cohort study in North Carolina found no association between lactational or prenatal *p,p*'-DDE exposures and self-reported Tanner genitalia stages in 278 boys aged 10-15 years (Gladen et al. 2000). The North Carolina study differed from ours in assessing gestational exposures and used self-reported Tanner staging, whereas we focused on prepubertal exposures and used physician-assessed staging including gonadal palpation and comparison with an orchidometer, more precise measures of gonadal development (Euling et al. 2008).

In a cross-sectional study of 887 Flemish boys aged 14-15 years living in an urban industrial area, boys with higher HCB levels attained maturity (Tanner stage 3+ for genitalia and pubic hair growth) significantly earlier (Den Hond et al. 2010). Unlike our longitudinal study in which OCPs were measured on average about 8 years prior to sexual maturation, the Flemish

study was cross-sectional (OCPs and puberty were assessed at the same time). Also, the Flemish study defined maturity as Tanner stage 3+ for genitalia and pubic hair growth, which is considered mid-puberty, whereas we defined maturity using Tanner stage 5 for genitalia, Tanner stage 5 for pubic hair growth, or TV≥20 mL. Discordant findings may also reflect different mixtures of industrial exposures in the two populations, and/or differences in serum concentration between the two cohorts; for example, average OCP concentrations in the Russian cohort were much higher than the Flemish boys (Russian vs. Flemish: HCB median of 158.5 vs. 22.8 ng/g lipid; *p,p'-DDE* median of 286.5 vs. 104 ng/g lipid) (Den Hond et al. 2010; Lam et al. 2013).

Because analyses using lipid-normalized measures rather than wet-weight measures adjusted for total serum lipids did not substantially change our findings, we chose to focus on the wet weight concentrations as lipid-normalization may introduce some bias into the estimates in some instances (Li et al. 2013; Schisterman et al. 2005). In analyses additionally adjusted for BMI and height z-scores, the associations were attenuated but the overall interpretation did not change for the associations of HCB and β-HCH with TV≥20 mL and Tanner stage 5 for genitalia growth. However, higher serum β-HCH and *p,p*'-DDE concentrations (Q4) were no longer associated with Tanner stage 5 for pubic hair growth after adjustment for these growth measures (see Supplemental Material, Table S1). This demonstrates the complex interrelationships between puberty and BMI and height, which may be on the causal pathway between OCPs and sexual maturity.

Furthermore, as these OCPs are moderately correlated, we also constructed models including two or three OCPs in the same model to evaluate the impact on associations. Most previously observed associations were attenuated when more than one OCP was included in a model. However, consistent with the robustness of HCB as a predictor of pubertal onset (Lam et al. 2014), and the apparent sensitivity of pubic hair maturation to OCP exposures in this analysis, associations of HCB with genital maturation (TV≥20 mL) and Tanner stage 5 for pubic hair growth remained, with the latter retaining statistical significance even after adjustment for β-HCH and/or *p,p*'-DDE.

Although masculinization of the genitalia and testicular growth are regulated by the HPG axis, there are subtle differences at the level of the testes. For instance, testicular growth during puberty primarily reflects spermatogenesis. This is driven by follicle stimulating hormone (FSH) from the pituitary, which is stimulated by gonadotropin releasing hormone from the hypothalamus, in combination with testosterone (Kronenberg et al. 2008; Zawatski and Lee 2013). This process promotes the maturation of the seminiferous tubules and spermatogenesis. Virilization of genitalia is mediated by testosterone which is produced by the Leydig cells under stimulation of luteinizing hormone from the pituitary (Kronenberg et al. 2008; Zawatski and Lee 2013). In contrast to our previous finding in this cohort of an association of higher concentrations of HCB with later pubertal onset based on testicular volume (but not genitalia), in the maturation analysis, we found an association with later maturation for both genitalia development and testicular growth. These data suggest that as puberty advances, HCB may interfere with both the maturation of the seminiferous tubules and spermatogenesis as well as the interstitial Leydig cells (Kronenberg et al. 2008; Zawatski and Lee 2013).

β-HCH is hypothesized to have estrogenic action based on evidence of finding testicular atrophy and nephrocalcinosis (typically seen only in females) in exposed male rodents (Van Velsen et al. 1986). β-HCH mimics the effects of estradiol without being an agonist for the estrogen receptor (ER) and activates the transcription of promoters containing ERs by an unknown mechanism (Massaad et al. 2002). HCB and *p,p*'-DDE disrupt androgen production and AR binding in animals (Hahn et al. 1989; Kelce et al. 1995; Ralph et al. 2003); however, it is unclear how β-HCH may affect androgen receptor (AR) activity. With the potential concentration of organochlorine pesticides in fat and androgen-producing endocrine glands (Foster et al. 1993; Schaefer et al. 2000), perhaps androgen production or androgen metabolism are affected, which could then impair sexual hair development (Randall 2008). These compounds could also be affecting AR binding at the site of action (Randall 2008). Though regulation of adrenarche is not well understood, we hypothesize that HCB may disrupt the production of sex steroids in the zona reticularis of the adrenals and impair activation or responsiveness to androgens at the tissue levels (Havelock et al. 2004; Zawatski and Lee 2013). Obtaining adrenal androgen measurements in our cohort will help elucidate the mechanism for the delay in pubarche.

A limitation of our study is that only a single serum measurement of OCPs was obtained at enrollment. However, OCP measurements were obtained at a sensitive peripubertal exposure window (Lemasters et al. 2000, Pryor et al. 2000). We are also limited in our ability to generalize HCB and β-HCH findings to populations with lower exposures. Serum HCB and β-HCH concentrations in these boys were among the highest observed among contemporary pediatric populations (Lam et al. 2013), with the 25th percentile for HCB almost eight times the median

value of U.S children (Patterson et al. 2009). Therefore, our reference category included boys with relatively high concentrations (i.e., HCB). It is well understood that dose-response relationships for EDCs may be non-linear (Birnbaum 2012), thus we reported our results in quartiles as a conservative approach so that potential non-linear relationships could be examined without making any assumptions about specific forms of the dose-response. The mechanisms by which these OCPs may affect sexual maturation are poorly understood, obtaining reproductive hormones in this cohort would provide insight into the underlying mechanisms. Additionally, while the onset of spermatogenesis (spermarche) was not a focus of this analysis, spermarche closely reflects the achievement of testis function during male puberty (Kulin et al. 1989; Schaefer et al. 1990). Obtaining data on spermaturia may contribute to a better understanding of the relationship between OCPs and sexual maturity as it may better predict the clinical stage of puberty (Schaefer et al. 1990).

The strengths of our study include a prospective design that followed a cohort of prepubertal boys to sexual maturity, using three established pubertal measures including a highly precise method of testicular volume determination, in a population with a range of OCP serum concentrations. Additionally, the retention rate was high, and there was minimal differential loss to follow-up by demographic factors. Finally, one physician conducted all pubertal assessments across the nine annual physical exam visits, thus eliminating inter-examiner variability (Carlsen et al. 2000).

Conclusion

Our novel findings add new evidence to the limited literature that suggests that prepubertal exposure to environmental OCPs at relatively high levels, specifically HCB and β-HCH, may affect age at sexual maturity in boys. Additional research is warranted to understand the implications of environmentally-induced shifts in age at pubertal onset and sexual maturity on reproductive as well as psychosocial health.

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Table 1. Characteristics of Participants in the Russian Children's Study with Serum Organochlorine Pesticide Measurements at Enrollment (ages 8-9 years).

Characteristic	Total boys (n=350)
Child Characteristics	Mean ± SD or N (%)
Growth Measurements	
Height (cm)	129.0 ± 6
Weight (kg)	26.6 ± 5.3
Body Mass Index (BMI)	15.9 ± 2.3
WHO Height z-score	0.12 ± 1.0
WHO BMI z-score	-0.17 ± 1.3
Birth and Neonatal History	
Birth Weight (kg)	3.3 ± 0.5
Gestational Age (wks)	39.0 ± 1.8
Preterm birth (gestational age <37 wks)	33 (9%)
Macronutrients	
Total calories (calories)	2695.7 ± 931.0
% carbohydrates	54.3 ± 6.6
% fat	34.2 ± 5.9
% protein	11.6 ± 1.6
Other Characteristics	
Blood lead levels ≥ 5 μg/dL	86 (25%)
Parental and Residential Characteristics	
Any household smoking during pregnancy	58 (17%)
Maternal age at son's birth (<25 yrs)	222 (63%)
Maternal age at menarche	13.3 ± 1.3
Biological father absent from household	123 (35%)
Maximum Parental Education	
High School or Less	29 (8%)
Jr College/Technical School	198 (57%)
University/Post-Graduate Training	121 (35%)
Household Income, USD per month	
<175	107 (31%)
175-250	88 (25%)
>250	154 (44%)

Percentages may not total 100% due to rounding.

Missing: Birth weight, n=1; Household smoking during pregnancy, n=5; Maternal age at son's birth, n=3; Maternal age at menarche, n=26; Parental education, n=2; Household income, n=1; Macronutrients, n=3

Table 2. Adjusted Mean Shifts in Age at Sexual Maturity (Months, 95% CIs) by Quartiles of Wet-Weight Serum OCP Concentrations Amongst 350 Russian Boys.

	G5 (n=347)[a]		TV ≥ 20 mL (n=349)[b]		P5 (n=350)[c]	
Serum OCP Quartile	**Mean Shift (months) and 95% CI**	**P-value**	**Mean Shift (months) and 95% CI**	**P-value**	**Mean Shift (months) and 95% CI**	**P-value**
			HCB[d]			
Q1 (low)	Reference		Reference		Reference	
Q2	2.78 (-2.53, 8.09)	0.31	3.05 (-1.72, 7.81)	0.21	4.43 (-1.28, 10.14)	0.13
Q3	5.64 (0.34, 10.94)	0.04	5.34 (0.57, 10.10)	0.03	11.20 (5.27, 17.13)	<0.001
Q4 (high)	3.71 (-1.59, 9.00)	0.17	5.01 (0.21, 9.82)	0.04	9.73 (3.78, 15.67)	0.001
P for trend		0.10		0.02		<0.001
			β-HCH[e]			
Q1 (low)	Reference		Reference		Reference	
Q2	2.18 (-3.12, 7.48)	0.42	0.10 (-4.68, 4.88)	0.97	1.17 (-4.63, 6.96)	0.69
Q3	5.69 (0.36, 11.02)	0.04	3.71 (-1.13, 8.54)	0.13	8.67 (2.61, 14.74)	0.01
Q4 (high)	3.71 (-1.65, 9.08)	0.17	3.63 (-1.27, 8.53)	0.15	5.99 (-0.08, 12.07)	0.05
P for trend		0.09		0.07		0.01
			***p,p*'-DDE[f]**			
Q1 (low)	Reference		Reference		Reference	
Q2	-1.68 (-6.98, 3.61)	0.53	-0.32 (-5.10, 4.47)	0.90	-0.30 (-6.19, 5.59)	0.92
Q3	-1.34 (-6.67, 3.99)	0.62	-0.09 (-4.89, 4.71)	0.97	1.67 (-4.29, 7.63)	0.58
Q4 (high)	2.52 (-2.92, 7.97)	0.36	2.45 (-2.49, 7.39)	0.33	6.19 (0.11, 12.27)	0.05
P for trend		0.37		0.35		0.04

G5: Tanner stage 5 for genitalia growth; P5: Tanner stage 5 for pubic hair growth

[a]G5 model adjusted for baseline covariates: boys' total serum lipids, macronutrients (total caloric intake, percent calories from dietary carbohydrates, fat; and protein); missing macronutrients, n=3

[b]TV ≥ 20 mL model adjusted for baseline covariates: boys' total serum lipids, birth weight, blood lead levels, biological father's absence from the household; missing birth weight, n=1

[c]P5 model adjusted for baseline covariates: boys' total serum lipids, biological father's absence from the household

[d]HCB wet-weight quartiles (Q1-Q4, pg/g serum): Q1, 169-516; Q2, 517-751; Q3, 752-1,156; Q4, 1,157-15,482

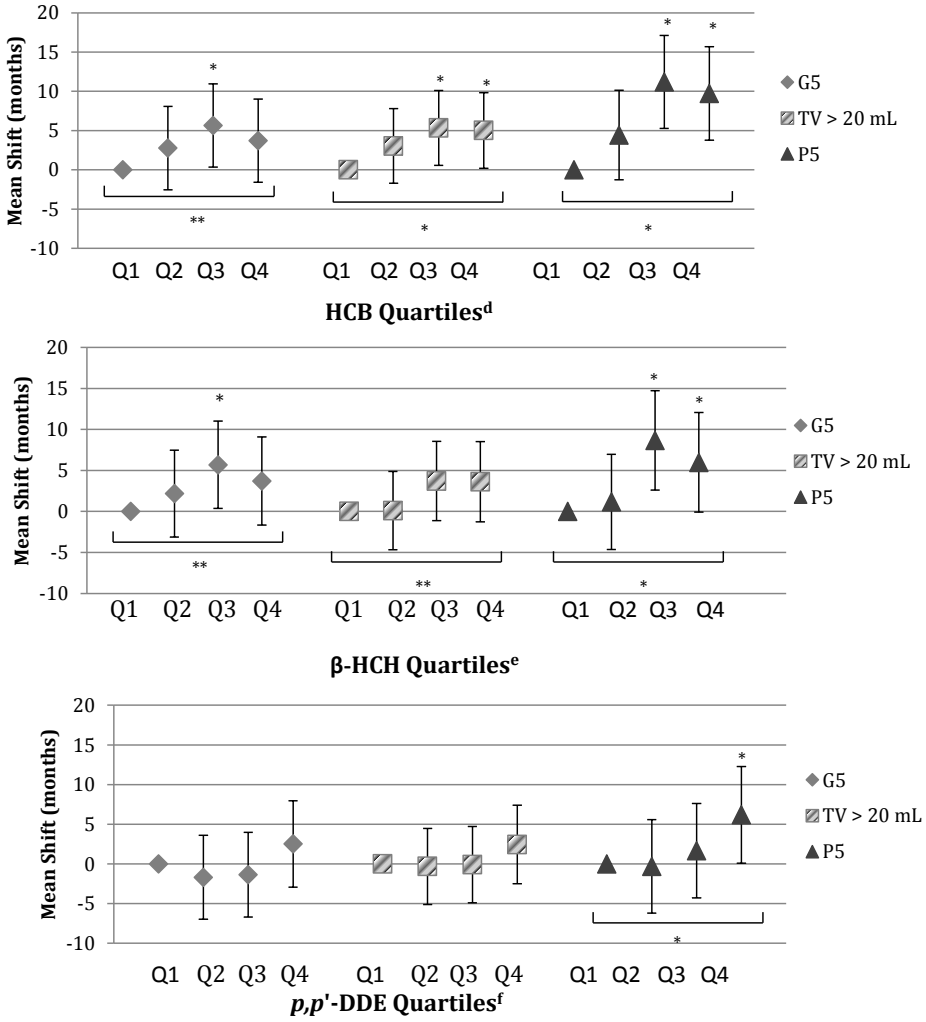
[e]β-HCH wet-weight quartiles (Q1-Q4, pg/g serum): Q1, 209-567; Q2, 568-814; Q3, 815-1,294; Q4, 1,295-13,732

[f]*p,p*'-DDE wet-weight quartiles (Q1-Q4, pg/g serum): Q1, 261-907; Q2, 908-1,406; Q3, 1,407-2,327, Q4, 2,328-41,301

Figure Legend

Figure 1. Adjusted mean shifts in age at sexual maturity (months, 95% CIs) by quartiles of wet-weight serum OCP concentrations among 350 Russian boys, relative to the lowest quartile (Q1). Baseline covariates for each model are G5: boy's total serum lipids, macronutrients (total caloric intake, percent calories from dietary carbohydrates, fat, protein) (missing macronutrients, n=3); TV ≥ 20 mL: boys' total serum lipids, birth weight, blood lead levels, biological father's absence from the household (missing birth weight, n=1); P5: boys' total serum lipids, biological father's absence from the household. HCB wet-weight quartiles (pg/g serum): Q1, 169–516, Q2, 517–751, Q3, 752–1,156, Q4, 1,157–15,482. β-HCH wet-weight quartiles (pg/g serum): Q1, 209–567, Q2, 568–814, Q3, 815–1,294, Q4, 1,295–13,732. *p,p*'-DDE wet-weight quartiles (pg/g serum): Q1, 261-907, Q2, 908-1,406, Q3, 1,407-2,327, Q4, 2,328-41,301. *p≤ 0.05 **p ≤ 0.10

Figure 1.


Mean Shift (months)
20
15
10
5
0
-5
-10
Q1 Q2 Q3 Q4 Q1 Q2 Q3 Q4 Q1 Q2 Q3 Q4
G5
TV > 20 mL
P5
HCB Quartiles[d]
β-HCH Quartiles[e]
p,p'-DDE Quartiles[f]