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Prepubertal organochlorine pesticide concentrations and age of pubertal onset among Russian boys

Thuy Lam [a,b], Paige L. Williams [c,d], Mary M. Lee [e,f], Susan A. Korrick [a,g], Linda S. Birnbaum [h], Jane S. Burns [a], Oleg Sergeyev [i,j], Boris Revich [k], Larisa M. Altshul [l,m], Donald G. Patterson Jr. [n,o,p], Wayman E. Turner [q], Russ Hauser [a,*]

[a] Environmental and Occupational Medicine and Epidemiology Program, Department of Environmental Health, Harvard School of Public Health, Boston, MA, USA
[b] Quintiles, Cambridge, MA, USA
[c] Department of Biostatistics, Harvard School of Public Health, Boston, MA, USA
[d] Department of Epidemiology, Harvard School of Public Health, Boston, MA, USA
[e] Pediatric Endocrine Division, Department of Pediatrics, University of Massachusetts Medical School, Worcester, MA, USA
[f] Department of Cell and Developmental Biology, University of Massachusetts Medical School, Worcester, MA, USA
[g] Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA
[h] National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Research Triangle Park, NC, USA
[i] Samara State Medical University, Department of Physical Education and Health, Samara, Russia
[j] Chapaevsk Medical Association, Chapaevsk, Samara Region, Russia
[k] Institute for Forecasting, Russian Academy of Sciences, Moscow, Russia
[l] Exposure, Epidemiology, and Risk Program, Department of Environmental Health, Harvard School of Public Health, Boston, MA, USA
[m] Environmental Health and Engineering, Inc., Needham, MA, USA
[n] EnviroSolutions Consulting, Inc., Auburn, GA, USA
[o] Axys Analytical Solutions, Sidney, BC, Canada
[p] Exponent, Inc., Maynard, MA, USA
[q] Centers for Disease Control and Prevention, Atlanta, GA, USA

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ABSTRACT

Background: In animal studies, organochlorine pesticide (OCP) exposure alters pubertal development; however, epidemiological data are limited and inconsistent.
Objective: To evaluate the associations of serum OCP concentrations [hexachlorobenzene (HCB), β-hexachlorocyclohexane (β-HCH), and *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE)] with male pubertal onset.
Methods: In Chapaevsk, Russia, a town environmentally contaminated with OCPs, 350 8–9 year old boys with measured OCPs were enrolled during 2003–2005 and were followed annually for eight years. We evaluated three measures of pubertal onset: testicular volume (TV) > 3 mL in either testis, or stage 2 or greater for genitalia (G2+), or pubic hair (P2+). We used multivariable interval-censored models to evaluate associations of OCPs (quartiles) with physician-assessed pubertal onset.
Results: In adjusted models, boys with higher HCB concentrations had later mean ages of TV > 3 mL and P2+ (but not G2+). Mean age at attaining TV > 3 mL was delayed 3.6 (95% CI: −2.6, 9.7), 7.9 (95% CI: 1.7, 14.0), and 4.7 months (95% CI: −1.4, 10.9) for HCB Q2, Q3, and Q4, respectively, compared to Q1 (trend p: 0.06). Boys with higher HCB concentrations reached P2+ 0.1 months earlier (95% CI: −5.8, 5.6) for Q2, 4.7 months later (95% CI: −1.0, 10.3) for Q3 and 4.6 months later (95% CI: −1.1, 10.3) for Q4 compared to Q1 (trend p: 0.04). There were no associations of serum β-HCH and *p,p′*-DDE concentrations with age of pubertal onset.
Conclusion: Higher prepubertal serum HCB concentrations were associated with later age of gonadarche and pubarche.

1. Introduction

Puberty is a dynamic process, characterized by rapid hormonal, physiologic, and behavioral changes including secondary sexual maturation and acceleration in linear and muscle mass growth (Giedd et al., 2006; Tanner and Whitehouse, 1976). Two hormonally distinct processes are involved in puberty: the maturation of the hypothalamic pituitary gonadal

Abbreviations: β-HCH, β-hexachlorocyclohexane; BLL, blood lead level; BMI, body mass index; G, genitalia Tanner stage; HCB, hexachlorobenzene; DDT, 1,1,1,-trichloro-2,2,bis(p-chlorophenyl)ethane; EDC, endocrine disrupting chemical; OCP, organochlorine pesticide; P, pubic hair Tanner stage; *p,p′*-DDE, *p,p′*-dichlorodiphenyldichloroethylene; PPS, preputial separation; TV, testicular volume measured with orchidometer.

* Corresponding author at: Environmental and Occupational Medicine and Epidemiology Program, Harvard School of Public Health, 665 Huntington Ave., Building 1, Room 1409, Boston, MA 02115, USA. Tel.: +1 617 432 3326.
E-mail address: rhauser@hsph.harvard.edu (R. Hauser).

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(HPG) system ("gonadarche") and adrenarche (Havelock et al., 2004; Kronenberg et al., 2008). These two pathways appear to be discretely regulated and activated by different cues (Havelock et al., 2004; Kronenberg et al., 2008). While androgens produced by either the adrenals or the testes can cause virilization of the genitalia and pubic hair, testicular enlargement indicates HPG activation. Pubic hair growth ("pubarche") is typically associated with adrenarche (Havelock et al., 2004). A number of factors including body composition, diet, and environmental exposures may interfere with gonadarche or pubarche (Golub et al., 2008; Kaplowitz, 2008; Mustanski et al., 2004).

Environmental chemicals that may affect male puberty include organochlorine pesticides (OCPs) such as hexachlorobenzene (HCB), β-hexachlorocyclohexane (β-HCH), and 1,1,1,-trichloro-2,2,bis(p-chlorophenyl)ethane (DDT), which were historically used as insecticides and fungicides (Barber et al., 2005; Breivick et al., 1999; Jaga and Dharmani, 2003). HCB and β-HCH can also be unintentional by-products of chlorinated chemicals manufacturing (Courtney, 1979; Jung et al., 1997). These lipophilic compounds as well as DDT's primary metabolite, p,p′-dichlorodiphenyldichloroethylene (*p,p′*-DDE), are highly stable, environmentally persistent, have long biological half-lives, and bioaccumulate through the food chain (Barber et al., 2005; Breivick et al., 1999; Jaga and Dharmani, 2003; Jung et al., 1997).

In rodents, fetal and peripubertal (post-natal day 21) *p,p′*-DDE exposure delayed male preputial separation (PPS) (Kelce et al., 1995), a marker of male puberty, and caused other male reproductive abnormalities (Ashby and Lefevre, 2000; Fry and Toone, 1981; Gray et al., 2001; Guillette et al., 1994; Kelce et al., 1995; Quinn et al., 2008). In contrast, *in utero* or post-natal HCB and β-HCH exposure caused reproductive and developmental abnormalities but did not affect the timing of PPS (Arnold et al., 1985; Courtney, 1979; Simon et al., 1979; Van Velsen et al., 1986). Epidemiologic evidence on the associations of OCPs and puberty is sparse and inconsistent. While in a North Carolina prospective study, measures of prenatal and lactational *p,p′*-DDE exposures were not associated with pubertal stage among boys aged 10–15 years (Gladen et al., 2000), a cross-sectional study of Flemish boys aged 14–15 years found an association of both serum *p,p′*-DDE and HCB concentrations with earlier pubic hair and genital development (Den Hond et al., 2010). To date, no studies on the association between β-HCH and pubertal development in children have been published. Given the suggestive animal evidence and the limited epidemiologic evidence on the relationship between OCPs and male puberty, we sought to assess this using a study design that improved upon methodological issues identified in prior studies.

Among Russian boys residing in a city with high environmental organochlorine contamination, we previously demonstrated that prepubertal serum dioxin concentrations were associated with a later pubertal onset defined as testicular volume (TV) > 3 mL (Korrick et al., 2011). In contrast, in the same cohort, maternal PCB concentrations measured at boys' study entry (age 8–9 years) were associated with earlier pubertal onset defined by Tanner genitalia stage 2 (G2) or higher (Humblet et al., 2011). In the present analysis, we examined the association of the boys' prepubertal serum concentrations of HCB, β-HCH, and *p,p′*-DDE with their age at pubertal onset.

2. Methods

2.1. Study population

The Russian Children's Study is an ongoing prospective cohort study of 499 boys in Chapaevsk, Russia, enrolled at age 8–9 years in 2003–2005, in a community with high environmental organochlorine contamination (Burns et al., 2012; Lam et al., 2013). A factory complex produced organochlorine compounds, including HCB, HCH and its derivatives (α, β, γ-HCH) but not DDT (Akhmedkanov et al., 2002). Briefly, 623 boys 8–9 years of age were identified from the town-wide health insurance system. Of these, 572 were eligible and 516 (90%) agreed to participate, although 17 boys were subsequently excluded because they were orphans (precluding collection of residential history and other information) (Burns et al., 2012; Lam et al., 2013). OCPs were not measured for the first 144 boys enrolled in the study, and five boys with severe chronic medical conditions that could affect growth were excluded from this analysis, leaving 350 of the original 499 boys with measured OCPs. 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.

The parent/guardian completed nurse-administered health and lifestyle questionnaires at entry on the child's birth, medical, and family history, physical activity, parental occupation and education, residential history, household income, and also completed a Russian Institute of Nutrition food frequency questionnaire to ascertain the child's dietary information; similar questionnaires were completed at annual study visits.

2.2. Physical examination and pubertal assessment

At study enrollment and annual visits thereafter, a standardized physical examination was conducted. Body mass index (BMI; kg/m^2) was calculated from measured height and weight. A single investigator (O.S.) performed annual pubertal assessments for up to eight follow-up visits according to a written protocol and without knowledge of the boys' OCP serum concentrations. TV was measured using a Prader orchidometer. Pubertal assessment for genitalia and pubic hair was based on a scale of 1 (immature) to 5 (sexually mature) by visual inspection according to established criteria (Tanner and Whitehouse, 1976). Pubertal onset was defined as stage 2 or higher for genitalia (G2+), or pubic hair growth (P2+), or TV > 3 mL for either testis.

2.3. Organochlorine exposure assessment

Fasting blood samples were collected from eligible boys at study entry and then centrifuged, aliquoted, and 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 C18 solid-phase extraction (SPE) followed by a multi-column automated cleanup and enrichment procedure using either large-volume (Turner et al., 1997) or small-volume SPE (Sjodin et al., 2004). Samples were analyzed with high-resolution mass spectrometry in selective ion monitoring mode (Barr et al., 2003). Total serum lipid content was determined from enzymatic measurements of total cholesterol and triglycerides (Phillips et al., 1989). Analytical coefficients of variation (CVs) for individual OCPs in QA/QC samples ranged between 10 and 15%. All OCP concentrations were above the limit of detection and were expressed as both a wet-weight (pg/g serum) and lipid-normalized (ng/g lipid).

2.4. Statistical analysis

Interval-censored survival analyses, both unadjusted and adjusted for potential confounders, were used to evaluate associations between boys' serum OCP concentrations (wet-weight concentrations categorized into quartiles with total serum lipids included as a covariate) and age of pubertal onset; each quartile was compared to the lowest quartile and tests for trend were performed by modeling OCP quartiles as an ordinal variable. We assumed a normal distribution for age at pubertal onset. Maximum likelihood estimates of regression parameters were obtained with the SAS LIFEREG procedure. The interval-censored approach assumed onset occurred in the interval between study visits (interval-censored), already occurred (left-censored), or had not yet