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Associations of Peripubertal Serum Dioxin and Polychlorinated Biphenyl Concentrations with Pubertal Timing among Russian Boys

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Short running title: Dioxins/PCBs and male puberty

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ABSTRACT

Background: Dioxins, furans, and polychlorinated biphenyls (PCBs), dioxin-like and nondioxin-like, have been linked to alterations in puberty.

Objectives: We examined the association of peripubertal serum levels of these compounds (and their toxic equivalents (TEQs)) with pubertal onset and maturity among Russian boys enrolled at ages 8–9 years and followed prospectively through ages 17-18 years.

Methods: At enrollment, 473 boys had serum dioxin-like compounds and PCBs measured. At the baseline visit and annually until age 17-18 years, a physician performed pubertal staging [Genitalia (G), Pubarche (P), and testicular volume (TV)]. 315 subjects completed the follow-up visit at 17-18 years of age. Pubertal onset was defined as $TV > 3$ mL, G2, or P2. Sexual maturity was defined as $TV \geq 20$ mL, G5, or P5. Multivariable interval-censored models were used to evaluate associations of lipid-standardized concentrations with pubertal timing.

Results: Medians (interquartile ranges) of the sum of dioxin-like compounds, TEQs, and nondioxin-like-PCBs were 362 pg/g lipid (279-495), 21.1 pg TEQ/g lipid (14.4-33.2), and 250 ng/g lipid (164-395), respectively. In adjusted models, the highest compared to lowest TEQ quartile was associated with *later* pubertal onset (months; 95% CI) [TV 11.6 (3.8, 19.4); G2 10.1 (1.4, 18.8)] and sexual maturity [TV 11.6 (5.7, 17.6); G5 9.7 (3.1, 16.2)]. However, the highest compared to the lowest quartile of nondioxin-like-PCBs, when co-adjusted by TEQs, was associated with *earlier* pubertal onset [TV -8.3 (-16.2, -0.3)] and sexual maturity [TV -6.3 (-12.2, -0.3); G5 -7.2 (-13.8, -0.6)]; the nondioxin-like-PCB associations were only significant when adjusted for TEQs. TEQs and PCBs were not significantly associated with pubic hair development.

Conclusions: Our results suggest that TEQs may delay, while nondioxin-like-PCBs advance, the timing of male puberty.

Introduction

Considerable evidence supports a decline in age of pubertal onset among girls in recent decades (Askglæde et al. 2009, Herman-Giddens et al. 1997, Sørensen et al. 2012), while studies in boys are limited with inconsistent findings (Herman-Giddens et al. 2012, Sørensen et al. 2012). The timing of puberty and associated physiological processes depends on neuroendocrine activation of the hypothalamic-pituitary-gonadal (HPG) axis (Havelock et al. 2004, Kronenberg et al. 2008). Developmental exposures to endocrine disrupting chemicals (Zoeller et al. 2012) are speculated to accelerate pubertal onset in girls (Parent et al. 2015, Zawatski and Lee 2013), although a recent study reported that exposures to some persistent organic pollutants were associated with later rather than earlier pubertal onset in 645 U.S. girls (Windham et al. 2015).

Organochlorines, such as dioxin-like compounds (DLCs: polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans, and dioxin-like polychlorinated biphenyls [PCBs]) and nondioxin-like-PCBs are examples of endocrine disruptors that alter pubertal timing in animal studies (Parent et al. 2015). Organochlorines are wide-spread environmental contaminants that are lipophilic and highly stable, with half-lives of many years (Milbrath et al. 2009). Consequently, even though the production of compounds like PCBs is banned, they persist in the environment, while dioxins are still generated as by-products of industrial chemical processes and incineration (UNEP 2009). Dioxin potency is assessed by relative affinity for the aryl hydrocarbon receptor (AhR), expressed as toxic equivalents (TEQ) (van den Berg et al. 2006).

In our previous publication on our longitudinal cohort of Russian boys, we reported follow-up to age 12 years (when 83% had pubertal onset) at which time there were associations of higher baseline serum TEQs, measured when the boys were 8-9 years, with later pubertal

onset by testicular volume (TV), and a suggestion of earlier onset by TV with higher nondioxin-like-PCBs (Korrick et al. 2011). We also reported that higher maternal serum nondioxin-like-PCBs, measured when the boys were age 8-9 years, were associated with earlier pubertal onset among these boys (Humblet et al. 2011). In our current analysis, which extends follow-up to age 17-18 years, all active participants have entered puberty and the majority (95%) have completed puberty, enabling us to evaluate the associations of DLCs, TEQs, and nondioxin-like-PCBs with both pubertal onset and sexual maturity.

Methods

Study Population.

The Russian Children's Study is a prospective cohort study of 499 boys residing in Chapaevsk, Russia, enrolled in 2003-2005 at ages 8-9 years and followed annually through 2012-2014 to ages 17-18 years for this analysis (Hauser et al. 2005). The study was approved by the Human Studies Institutional Review Boards of the Chapaevsk Medical Association, Harvard T. H. Chan School of Public Health, University of Massachusetts Medical School, and Brigham and Women's Hospital. The parent/guardian provided informed consent and the boys signed assent forms before participation. For this analysis, 10 boys in the original cohort were excluded due to chronic illnesses that could affect puberty. Of the remaining 489 subjects, 473 (97%) with baseline DLC and nondioxin-like-PCB measurements were included.

At study entry, each boy's parent or guardian completed nurse-administered health and lifestyle questionnaires (Lee et al. 2003), ascertaining birth and medical history, and demographic and socioeconomic status (SES) information. A validated Russian Institute of

Nutrition semi-quantitative food frequency questionnaire was used to characterize each child's diet (Martinchik et al. 1998, Rockett et al. 1997).

Physical Examination and Pubertal Assessment

At study entry and annual follow-up visits, a standardized anthropometric examination was performed by a single trained research nurse and pubertal staging was performed by a single physician (O.S.) without knowledge of serum organochlorine concentrations. Staging of genitalia (G) and pubic hair (P) as 1 (immature) to 5 (sexually mature) was by visual inspection (Tanner and Whitehouse 1976). TV was measured using a Prader orchidometer. Pubertal onset was defined as G2, P2, or TV>3 mL for either testis. Sexual maturity was defined as G5, P5 or at least one testis ≥ 20 mL (Joustra et al. 2015).

Exposure Assessment

Fasting blood samples were collected at ages 8-9 years, and serum stored at -35°C until shipment on dry ice to the U.S. Centers for Disease Control and Prevention, Atlanta, GA and analyzed during 2004-2008. The samples, including method blanks and quality control samples, were analyzed for 7 dioxins, 10 furans, 41 PCBs and total lipids as described previously (Burns et al. 2009). Values below the limit of detection (LOD) were assigned a value equal to the LOD divided by the square root of 2 (Baccarelli et al. 2005). Organochlorine concentrations were expressed as lipid-standardized concentrations (wet-weight levels divided by lipid concentrations) or as the lipid-standardized sum of the toxic-equivalency factor (TEF)-weighted dioxin-like concentrations (TEQs).

Statistical Analysis

Unadjusted and adjusted interval-censored survival analyses were used to evaluate the associations of boys' baseline sum of DLC (Σ DLC), TEQ (Σ TEQ), and nondioxin-like-PCB (Σ Nondioxin-like-PCB) congener concentrations (in quartiles) with age at pubertal onset and sexual maturity. Ages at pubertal onset and sexual maturity were assumed to follow a normal distribution. Trend tests were conducted by modeling quartiles as an ordinal variable. The interval-censored model allows for pubertal onset and sexual maturity to occur between study visits (interval-censored), before the study entry visit (left-censored), or after the last study visit (right-censored) (Christensen et al. 2010; Lindsey and Ryan 1998). Parameter estimates using maximum likelihood methods were implemented via PROC LIFEREG in SAS Version 9.2 (SAS Institute, Cary NC).

All covariates except height, body mass index (BMI), and parental pubertal milestones were identified as *a priori* predictors of pubertal development and were considered for inclusion in the models (Table 1). A separate model was fit for each measure of pubertal onset and sexual maturity. A core model was developed by first evaluating associations of each covariate with pubertal onset and sexual maturity and retaining those with $p < 0.20$, then including these in a full model and using backwards selection (likelihood ratio test) to exclude covariates with $p > 0.10$. To check for confounding, covariates with $p < 0.20$ were added individually to the final model and those associated with $\geq 10\%$ change in organochlorine trend test coefficients retained. Statistical significance was defined as $p \leq 0.05$. Missing covariate data was addressed using a complete-case analysis.

We and others have shown that dioxin-like compounds and nondioxin-like-PCBs have divergent associations with puberty (Humblet et al. 2011; Korrick et al. 2011; reviewed in Zawatski and Lee 2013), therefore we adjusted our final models for both simultaneously. We also assessed the robustness of the associations in single organochlorine models. Previously, we reported an association of nondioxin-like-PCBs with BMI and height (Burns et al. 2011) in this cohort suggesting that these measures may mediate or confound the associations with puberty; therefore, we excluded baseline BMI and height from the primary analysis. We conducted sensitivity analyses adjusting for BMI and height z-scores (World Health Organization standards) (de Onis et al. 2007). Sensitivity analyses were also conducted to assess robustness of associations after further adjustment for maternal age at menarche (available for 92% of participants).

Results

Study population

Table 1 summarizes perinatal history, and baseline anthropometric measurements, diet, maternal, and household characteristics, serum organochlorine concentrations, and missing data, which was minimal. The boys were, on average, within the normal range for height and BMI (de Onis et al. 2007). Correlations among the organochlorine concentrations ranged from 0.74-0.82 (data not shown). Baseline characteristics between those boys with (n=473) versus without (n=26) organochlorine measurements were similar (Burns et al. 2009). 315 boys completed annual follow-up visits through age 17-18 years (67% retention rate). Although serum organochlorines, height z-scores, and most demographic characteristics did not differ significantly between boys who remained in the study compared to those who dropped out before the 17-18 year visit (data not shown), boys who remained in the study were leaner at baseline

(mean BMI z-score -0.34 vs. -0.05), and more likely to have post-secondary school educated parents (95% vs. 87%).

Pubertal Onset and Sexual Maturity

At study entry (age 8 to 9 years), pubertal onset had occurred in 9% (P2), 30% (G2), and 14% (TV>3 mL). At age 17-18 years, all 315 boys had entered puberty and 74% (P5), 98% (G5), and 97% (TV \geq 20mL) were sexually mature.

Pubertal onset and organochlorines

In models co-adjusted for serum Σ TEQs and Σ nondioxin-like-PCBs (Table 2), higher quartiles of Σ TEQs were associated with later pubertal onset, with a monotonic increase in TV delay with increasing quartiles and an apparent plateau for genitalia (Table 2). In contrast, the highest Σ Nondioxin-like-PCB quartile compared to the lowest was associated with *earlier* pubertal onset by TV, with a significant trend test. In models co-adjusted for serum Σ DLCs and Σ nondioxin-like-PCBs, the highest two quartiles of Σ DLCs as compared to the first quartile were also significantly associated with later pubertal onset by TV, but not genitalia. None of the organochlorines were significantly associated with age of onset of pubarche.

In single organochlorine models (see Supplemental Material, Table S1), the association of Σ TEQs with later pubertal onset by TV remained significant although attenuated, e.g. 5.6 months (95% CI 0.3, 10.9) for the highest vs. lowest quartile, compared with 11.6 months (95% CI 3.8, 19.4) when adjusted for Σ nondioxin-like-PCBs. The associations between TEQs and genitalia, and between Σ DLCs and TV, were attenuated, although the trend test for Σ DLCs and TV was significant. However, when modeled alone, the associations between Σ nondioxin-like-PCBs and earlier pubertal onset by TV were null, e.g. approximately 0 months.

Sexual maturity and serum OC concentrations

In models co-adjusted for Σ TEQs and Σ nondioxin-like-PCBs, higher Σ TEQs were associated with a monotonic dose-response association for *later* sexual maturity by TV and genitalia. However, the highest quartile of Σ nondioxin-like-PCBs compared to the lowest was associated with *earlier* sexual maturity by TV and genitalia (Table 3). In models co-adjusted for Σ DLCs and Σ nondioxin-like-PCBs, the highest two quartiles of Σ DLCs were significantly associated with a delay in sexual maturity by TV but not genitalia. Both higher Σ TEQs and Σ DLCs were nonsignificantly associated with a nonlinear delay in pubic hair maturation, with only the association of quartile 2 of Σ DLCs significant. Associations between Σ nondioxin-like-PCBs and mature pubic hair were null.

In single organochlorine models (see Supplemental Material, Table S2), the associations of higher Σ TEQs with later sexual maturity remained significant although attenuated, e.g. for TV 7.7 months (95% CI 3.6, 11.8) for the highest vs. lowest quartile, compared with 11.6 months (95% CI 5.7, 17.6) when adjusted for Σ nondioxin-like-PCBs. Also, without adjustment for Σ nondioxin-like-PCBs, the association between Σ DLCs and TV was minimally attenuated. In contrast, without adjustment for Σ TEQs, the association between Σ nondioxin-like-PCBs and earlier sexual maturity became null (see Supplemental Material, Table S2).

Sensitivity Analyses

In multiple organochlorine models adjusted for baseline height and BMI z-scores, associations of Σ TEQs and Σ DLCs with pubertal onset and sexual maturity were similar to models not adjusted for height and BMI z-scores (see Supplemental Material, Tables S3 and S4), whereas, the associations of Σ nondioxin-like-PCBs with pubertal onset and sexual maturity by

TV and genitalia were strengthened (Figures 1 and 2). All associations changed minimally after adjustment for mother's age at menarche (data not shown).

Discussion

The few epidemiological studies that have examined the associations of dioxins and PCBs with male pubertal timing have yielded inconsistent results (Croes et al. 2014, Den Hond et al. 2002, Den Hond et al. 2011, Grandjean et al. 2012, Korrnick et al. 2011, Leijds et al. 2008). In our longitudinal cohort, we examined associations among a wide range of peripubertal serum dioxin-like compounds and nondioxin-like-PCB concentrations with male pubertal onset and sexual maturity. In models with multiple organochlorines (i.e., co-adjustment), we found robust associations of dioxin-like compounds and TEQs with later pubertal onset and sexual maturity while nondioxin-like-PCBs were associated with earlier pubertal onset and sexual maturity.

In models co-adjusted for serum Σ nondioxin-like-PCBs, the highest as compared to the lowest quartile of baseline serum Σ TEQs, the axiomatic measure of dioxin AhR toxicity, were associated with later pubertal onset and sexual maturity by TV and genitalia staging by almost a year. Similar to our results after three years of follow-up (Korrnick et al. 2011), the current findings were robust in sensitivity analyses adjusting for baseline BMI and height z-scores or mother's age at menarche (data not shown). Whereas the associations of Σ DLCs with TV were similar (albeit of a lesser magnitude) to those observed for Σ TEQs, Σ DLCs were not associated with genitalia stage. If causal, this may be consistent with a differential impact of AhR-mediated functions on TV as compared with other aspects of genital maturation. Our observations of nonlinear associations of Σ DLCs with pubic hair growth may be impacted by imprecise pubic

hair staging because of more missing data for this measure due to shaving pubic hair in later adolescence.

Rodent studies suggest that the potent dioxin, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), is associated with delayed preputial separation (Bell et al. 2007, Hamm et al. 2003, Theobald et al. 2003), an androgen-dependent indicator of male pubertal onset. Few epidemiological studies have measured serum dioxins in children due in part to the large serum volume required and high cost; therefore, there is little human comparative data. A Dutch study, limited by a small sample size of 15 adolescent boys and concurrent low serum TEQs (median 1.5 TEQ pg/g lipid), found no association between prenatal, lactational or cross-sectional measures of serum TEQs and pubertal stage, although boys with higher cross-sectional serum TEQs reported later age at first ejaculation (Leijs et al. 2008). A Belgian group reported that living near dioxin-emitting waste incinerators as a proxy measure of exposures was associated with later pubertal maturation among 80 boys (Den Hond et al. 2002). However, both that study and later cross-sectional studies using an indirect luciferase reporter measure of AhR activity (CALUX), found no association with pubertal staging (Den Hond et al. 2002, Den Hond et al. 2011, Croes et al. 2014). Other explanations for these null findings may be due to indirect TEQ measurement, relatively low exposure levels, or cross-sectional design.

In contrast to our findings regarding Σ TEQs and later pubertal timing, higher peripubertal serum Σ non-dioxin-like-PCBs concentrations, co-adjusted for TEQs, were associated with earlier pubertal onset by TV, and earlier sexual maturity by TV and genitalia (Tables 2 and 3). Among boys, longitudinal studies have linked clinically delayed puberty to osteopenia (Finkelstein et al. 1996) and earlier puberty to increased risk of adult obesity (van Lenthe et al. 1996) and

cardiovascular disease (Prentice and Viner 2013). Alterations in timing of puberty have also been associated with psychosocial and behavior issues (Graber 2013). In both our current and prior analyses (Korrick et al. 2011), we did not observe associations between Σ nondioxin-like-PCBs and puberty in models unadjusted for Σ TEQs (See Supplemental Tables 1 and 2). We ascribe this to the positive correlation between Σ TEQs and Σ nondioxin-like-PCBs ($r=0.82$), and the strong association of Σ TEQs with *later* pubertal timing which may confound the effects of Σ nondioxin-like-PCBs. Thus we believe that it is necessary to include both classes of organochlorines in models assessing their associations with pubertal development. Our analytic approach was further justified by the fact that, despite correlation between measures, there was no major influence on the precision of the effect estimates for TEQs or nondioxin-like-PCBs in models including both. However, given that the biological mechanism underlying the effects of nondioxin-like-PCBs on puberty is unknown, the observed associations with puberty should be interpreted with caution.

In contrast to our assessment of *peripubertal* concentrations, longitudinal studies of 244 U.S. boys (Gladen et al. 2000) and 60 Taiwanese boys (Hsu et al. 2005) that assessed *prenatal* serum nondioxin-like-PCB concentrations did not find associations with timing of male pubertal development. However, among 394 Faroese boys, higher prenatal serum nondioxin-like-PCB concentrations were associated with a significant trend towards later pubertal development among boys at age 14 years (Grandjean et al. 2012).

Three cross-sectional Belgium studies that examined the association of peripubertal serum nondioxin-like-PCB (PCBs 138, 153, and 180) concentrations with male pubertal development by Tanner staging reported inconsistent results. While the initial study found later

pubertal development with higher serum nondioxin-like-PCBs among 80 boys (Den Hond et al. 2002), subsequently, a much larger study (n=887) reported a doubling of serum nondioxin-like-PCBs associated with earlier puberty (Den Hond et al. 2011). The most recent study of 324 14-15 year old boys found no association between higher serum nondioxin-like-PCBs and pubertal timing, although higher nondioxin-like-PCBs were associated with higher serum testosterone and sex hormone-binding globulin and lower estradiol concentrations (Croes et al. 2014). In the interval between these two later studies (Den Hond et al. 2011 and Croes et al. 2014), serum nondioxin-like-PCB concentrations had decreased 23%, suggesting that null findings in Croes et al. might reflect differences in exposure over time.

The inconsistent results between our study and both the earliest (Den Hond et al. 2002) and most recent (Croes et al. 2014) Belgian studies may be due to smaller sample size and lower serum concentrations, respectively. For example, the geometric mean (95% CI) of summed PCBs 138, 153, and 180 among the Russian boys was double that observed in Croes et al. (Croes et al. 2014), 103.7 (97.4, 110.3) ng/g lipid versus 49.6 (45.7, 53.8) ng/g lipid. Differences in study design (cross-sectional versus longitudinal), the unique PCB congener profile in a given community, as congeners have different estrogenic, androgenic, and anti-estrogenic properties (ATSDR 2000), and confounding by co-exposures to other endocrine disruptors are other factors that might also contribute to the inconsistent findings.

Puberty depends on a complex interplay between the central neuroendocrine system and the gonads modulated by a sex-steroid mediated negative feedback mechanism. The secretion of neuroendocrine factors stimulates the pulsatile secretion of gonadotropin releasing hormone (GnRH), which in turn triggers pituitary secretion of luteinizing hormone (LH) and follicle

stimulating hormone (FSH) (Ebling et al. 2005, Ojeda et al. 2010). LH stimulates testicular Leydig cells to produce testosterone and FSH promotes the maturation of the seminiferous tubules. In concert with maturation of the HPG axis, the adrenal zona reticularis increases adrenal androgen production.

Dioxin-like compounds and PCBs can potentially perturb reproductive system homeostasis via various mechanisms, such as activation or interference with the HPG axis or inhibition of testosterone biosynthesis (Parent et al. 2015, Svechnikov et al. 2010). Experimental data show that dioxins can suppress GnRH secretion and delay pubertal onset via an AhR pathway (Parent et al. 2015, Mueller et al. 2014). Dioxins may also have testicular actions as the AhR is widely expressed in testes (Schultz et al. 2003) and its signaling pathway has extensive crosstalk with steroid hormone receptors (Brokken et al. 2014, Schultz et al. 2003).

We reported previously that higher prepubertal serum Σ non-dioxin-like-PCBs concentrations were associated at ages 11-12 years with lower height and BMI (Burns et al. 2011) and that baseline height and BMI z-scores were associated with earlier pubertal development (Hauser et al. 2008, Korrick et al. 2011). After adjustment in our sensitivity analyses for baseline height and BMI z-scores, associations between Σ non-dioxin-like-PCBs and earlier pubertal development were strengthened, reflecting height and BMI acting as negative confounders rather than as mediators.

We regarded our measurement of serum organochlorines at ages 8-9 years as indicative of childhood exposures, although given their long biological half-lives, these levels may also reflect perinatal exposures (Verner et al. 2015). This partially limits our ability to conclude that childhood exposures are solely responsible for the associations we observed.

Our Russian Children's Study is one of the few prospective cohort studies to follow boys from prepuberty to sexual maturity with annual physician assessments of puberty using both Tanner staging and measurement of testicular volume, a more precise assessment than visual inspection (Biro et al. 1995). This provides the unique advantage of having more accurate data on the full pubertal process, from onset through sexual maturity. For most study participants, serum organochlorines were measured before pubertal onset, reducing the likelihood of reverse causation impacting our findings. Additionally, physical assessments were performed without knowledge of the boy's serum organochlorine concentrations, and our analysis was adjusted for many potential confounders. Moreover, our cohort was fairly large with a high retention rate and we found little difference between those who remained on study and those who were lost to follow-up, reducing potential bias. Our study design and analytic approach enabled us to include the observed data from all 473 boys, not just the 315 boys who completed the ninth year of follow-up, also reducing potential bias. Lastly, our cohort is characterized by exposure to multiple, correlated organochlorines. Given our comprehensive organochlorine measurements, and our prior results (Humblet et al. 2011, Korrick et al. 2011) suggesting opposing effects, we felt the most appropriate and conservative statistical approach to assess the independent effects of TEQs and nondioxin-like-PCBs on pubertal timing was to include both in the models.

In conclusion, among boys with higher peripubertal serum levels of Σ TEQs or Σ nondioxin-like-PCBs, we observed differences in timing of puberty as compared with their less exposed peers. These findings suggest that the prepubertal window may be vulnerable to organochlorine disruption of male pubertal development. Moreover, the divergent associations we observed between Σ TEQs and Σ nondioxin-like-PCBs with later and earlier pubertal timing, respectively, may suggest effects are mediated via different biologic mechanisms. The potential

impact of these compounds on pubertal timing has implications for both adolescent and adult health, and is a critical public health concern.

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Table 1: Descriptive characteristics of 473 boys with serum organochlorine measurements at entry into the Russian Children's Study during 2003-2005

Physical Characteristics – Mean (SD)	
Height z-score ^a	0.12 (1.00)
BMI z-score ^a	-0.19 (1.26)
Prenatal and Birth History – Median (25 th , 75 th -iles) ^b	
Mother's age at son's birth	22 (20, 26)
Birthweight (kg)	3.4 (3.1, 3.7)
Gestational age (wks)	40 (38, 40)
Prenatal tobacco smoke exposure (N, %)	224 (49%)
Prenatal alcohol consumption (N, %)	59 (13%)
Boys Daily Dietary Intake – Median (25 th , 75 th -iles) ^b	
Total calories (calories)	2674 (2092, 3443)
Percent calories from carbohydrates	55 (50, 59)
Percent calories from fat	34 (30, 37)
Percent calories from protein	11 (10, 12)
Boys Alcohol Consumption (beer, liquor) (N, %) ^b	274 (58%)
Boys Daily Physical Exercise Level (N, %) ^b	
None	129 (27%)
< 2 hours/day	142 (30%)
≥ 2 hours/day	201 (43%)
Household Characteristics (N, %) ^b	
Parental education – maximum	
Secondary education or less	37 (8%)
Junior college/technical training	279 (59%)
University graduate	153 (33%)
Household income	
< 175 U.S. dollars/month	164 (35%)
175 – 250 U.S. dollars/month	123 (26%)
> 250 U.S. dollars/month	185 (39%)
Biological father resides in home	310 (66%)
Serum Organochlorine Compounds – Median (25 th , 75 th -iles) ^b	
ΣDioxin-like Compounds (pg/g lipid) ^c	362 (279, 495)
ΣToxic Equivalents (pg TEQ/g lipid) ^d	21.1 (14.4, 33.2)
ΣNondioxin-like Polychlorinated Biphenyls (ng/g lipid) ^e	250 (164, 395)

^a WHO age-adjusted z-scores: <http://www.who.int/childgrowth/en/>;

^b missing information: mother's age at son's birth (n=5), birth weight (n=3), gestational age (n=4), prenatal tobacco smoke (n=11), prenatal alcohol consumption (n=16), dietary information (n=3), boys alcohol consumption (n=15), physical activity (n=1), parental education (n=4), household income (n=1), TEQs (n=5), nondioxin-like-PCBs (n=5).

^c Dioxin-like compounds includes dioxins (TCDD, 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, 1,2,3,4,6,7,8-HpCDD, OCDD), furans (2,3,7,8-TCDF, 1,2,3,7,8-PeCDF,

2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, 1,2,3,7,8,9-HxCDF, 2,3,4,6,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,7,8,9-HpCDF, OCDF), and PCBs (IUPAC congeners 77, 81, 126, 169).
^dTEQs includes dioxin-like compounds (see above) and additional PCBs (IUPAC congeners 105, 118, 156, 157, 167, 189).

^e Nondioxin-like PCBs (IUPAC congeners: 18, 28, 52, 49, 44, 74, 66, 101, 99, 87, 110, 118, 105, 151, 149, 146, 153, 138/158, 128, 167, 156, 157, 178, 187, 183, 177, 172, 180, 170, 189, 201, 196/203, 195, 194, 206)

Table 2. Adjusted mean shifts in age at pubertal onset [months (95% CI)] by quartiles of serum dioxin-like compounds (DLCs), toxic equivalents (TEQs), and nondioxin-like polychlorinated biphenyls (PCBs) among 473 Russian boys, enrolled at ages 8-9 years and followed to 17-18 years

Serum Quartile	Testicular Volume > 3 mL ^a		Genitalia Stage ≥ 2 ^b		Pubarche Stage ≥ 2 ^c	
	Mean shift (95% CI)	P-value	Mean shift (95% CI)	P-value	Mean shift (95% CI)	P-value
ΣTEQs, adjusted for Σnondioxin-like-PCBs ^d						
Q1	Reference		Reference		Reference	
Q2	4.0 (-1.9, 9.8)	0.19	8.1 (1.5, 14.7)	0.02	3.7 (-2.7, 10.1)	0.26
Q3	7.5 (0.6, 14.4)	0.03	10.1 (2.3, 17.9)	0.01	4.8 (-2.8, 12.4)	0.22
Q4	11.6 (3.8, 19.4)	0.004	10.1 (1.4, 18.8)	0.02	3.3 (-5.3, 11.8)	0.46
Trend test ^e		0.003		0.03		0.45
ΣDLCs, adjusted for Σnondioxin-like-PCBs ^e						
Q1	Reference		Reference		Reference	
Q2	1.3 (-4.3, 7.0)	0.64	3.8 (-2.5, 10.1)	0.24	2.5 (-3.7, 8.7)	0.43
Q3	6.8 (0.6, 13.0)	0.03	6.5 (-0.4, 13.5)	0.07	2.5 (-4.3, 9.2)	0.48
Q4	8.1 (1.1, 15.1)	0.02	0.4 (-7.5, 8.3)	0.92	-6.4 (-14.0, 1.2)	0.10
Trend test ^e		0.01		0.71		0.16
ΣNondioxin-like-PCBs, adjusted for ΣTEQs ^f						
Q1	Reference		Reference		Reference	
Q2	-3.1 (-8.9, 2.8)	0.31	0.4 (-6.2, 7.0)	0.91	-0.9 (-7.3, 5.6)	0.79
Q3	-5.6 (-12.3, 1.2)	0.11	-4.4 (-11.9, 3.2)	0.26	-5.2 (-12.7, 2.2)	0.17
Q4	-8.3 (-16.2, -0.3)	0.04	-5.3 (-14.1, 3.5)	0.24	-2.3 (-11.0, 6.5)	0.61
Trend test ^e		0.04		0.18		0.45

Interval-censored survival models: ^aadjusted for birthweight, household income, dietary fat intake, boy's alcohol intake, boy's daily exercise; ^badjusted for birthweight, biological father living in home, parental education, daily caloric intake, boy's alcohol intake; ^cadjusted for prenatal alcohol intake, biological father living in home, daily caloric and protein intake.

^dΣTEQ quartiles: Q1 4.0 – 14.5; Q2 14.6 – 21.0; Q3 21.1 – 33.2; Q4 33.3 – 174.7 pg/g lipid;

^eΣDLC quartiles: Q1 122 – 280; Q2 281 – 366; Q3 367 – 486; Q4 487 – 2963 pg/g lipid;

^fΣNondioxin-like-PCB quartiles: Q1 62 – 166; Q2 167 – 249; Q3 250 – 396; Q4 397 – 4248 ng/g lipid.

^gtrend tests performed by modeling OC quartiles as an ordinal variable.

Table 3. Adjusted mean shifts in age at sexual maturity [months (95% CI)] by quartiles of serum dioxin-like compounds (DLCs), toxic equivalents (TEQs), and nondioxin-like polychlorinated biphenyls (PCBs) concentrations among 473 Russian boys, enrolled at ages 8-9 years and followed to 17-18 years

Serum Quartile	Testicular Volume \geq 20 mL ^a		Genitalia Stage 5 ^b		Pubarche Stage 5 ^c	
	Mean shift (95% CI)	P-value	Mean shift (95% CI)	P-value	Mean shift (95% CI)	P-value
Σ TEQs, adjusted for Σ nondioxin-like-PCBs ^d						
Q1	Reference		Reference		Reference	
Q2	6.0 (1.6, 10.5)	0.008	4.4 (-0.5, 9.3)	0.08	4.9 (-1.1, 10.9)	0.11
Q3	8.8 (3.7, 14.0)	<0.001	7.5 (1.9, 13.2)	0.009	4.5 (-2.6, 11.5)	0.22
Q4	11.6 (5.7, 17.6)	<0.001	9.7 (3.1, 16.2)	0.004	5.5 (-2.6, 13.6)	0.18
Trend test ^e		<0.001		0.004		0.24
Σ DLCs, adjusted for Σ nondioxin-like-PCBs ^e						
Q1	Reference		Reference		Reference	
Q2	1.4 (-3.0, 5.8)	0.53	-0.6 (-5.5, 4.3)	0.80	6.8 (0.9, 12.8)	0.03
Q3	4.9 (0.2, 9.7)	0.04	1.1 (-4.2, 6.4)	0.68	6.2 (-0.1, 12.5)	0.06
Q4	5.5 (-0.01, 11.0)	0.05	0.2 (-6.0, 6.3)	0.96	2.5 (-4.8, 9.8)	0.50
Trend test ^e		0.04		0.86		0.52
Σ Nondioxin-like-PCBs, adjusted for Σ TEQs ^f						
Q1	Reference		Reference		Reference	
Q2	-5.0 (-9.4, -0.6)	0.03	-2.9 (-7.8, 2.0)	0.24	3.4 (-2.6, 9.5)	0.27
Q3	-3.0 (-8.1, 2.1)	0.25	-2.1 (-7.7, 3.6)	0.47	1.5 (-5.4, 8.4)	0.67
Q4	-6.3 (-12.2, -0.3)	0.04	-7.2 (-13.8, -0.6)	0.03	1.0 (-7.3, 9.2)	0.82
Trend test ^e		0.06		0.07		0.92

Interval-censored survival models: ^aadjusted for birthweight, biological father living in home, parental education; ^badjusted for mother's age at son's birth, household income, daily caloric intake, boy's daily exercise; ^cadjusted for prenatal tobacco smoke, biological father living in home.

^d Σ TEQ quartiles: Q1 4.0 – 14.5; Q2 14.6 – 21.0; Q3 21.1 – 33.2; Q4 33.3 – 174.7 pg/g lipid;

^e Σ DLC quartiles: Q1 122 – 280; Q2 281 – 366; Q3 367 – 486; Q4 487 – 2963 pg/g lipid;

^f Σ Nondioxin-like-PCB quartiles: Q1 62 – 166; Q2 167 – 249; Q3 250 – 396; Q4 397 – 4248 ng/g lipid.

^gtrend tests performed by modeling OC quartiles as an ordinal variable.

Figure Legends

Figure 1. Adjusted mean shifts in age at pubertal onset [months (95% CI)] by quartiles of peripubertal serum nondioxin-like polychlorinated biphenyl concentrations by testicular volume (A) and genitalia (B), unadjusted and adjusted for baseline body mass index and height z-scores.

Figure 2. Adjusted mean shifts in age at sexual maturity [months (95% CI)] by quartiles of peripubertal serum nondioxin-like polychlorinated biphenyl concentrations by testicular volume (A) and genitalia (B), unadjusted and adjusted for baseline body mass index and height z-scores.

Figure 1.

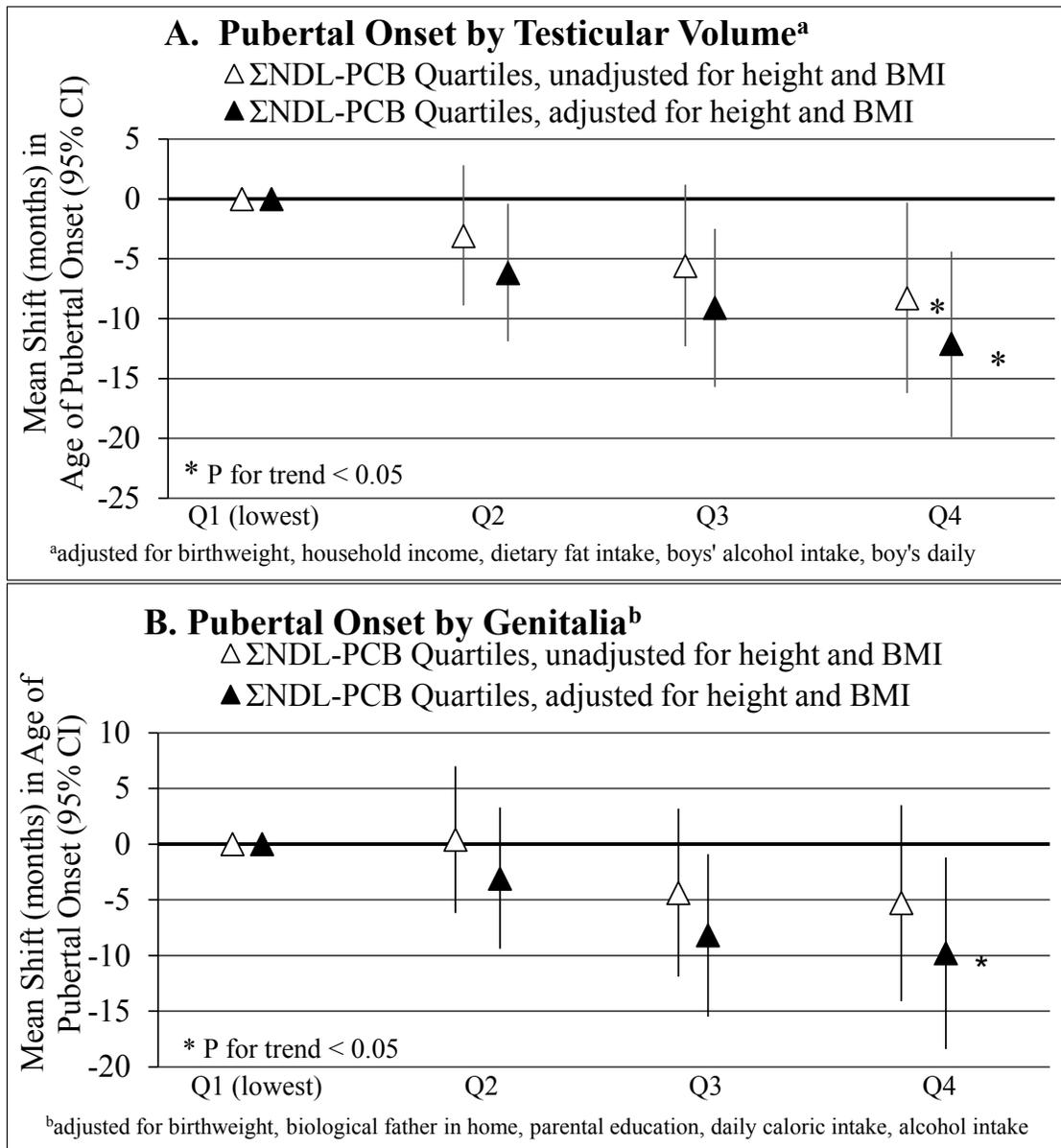


Figure 2.

