Peripubertal Serum Organochlorine Concentrations and Longitudinal Growth to Young Adulthood among Russian Boys

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Introduction

Chapaevsk (population 72,000) is a city in central Russia that has been the site of chemical production. In the past, the Middle Volga Chemical Plant produced organochlorine industrial and agricultural chemicals, e.g. the pesticides hexachlorobenzene (HCB) and beta-hexachlorocyclohexane (βHCH), and, as by-products, the dioxin-like compounds (DLCs) polychlorinated dibenzo-*p*-dioxins and dibenzofurans. These compounds contaminated the city's air, soil, water and food supply, including locally raised animals and vegetables, and led to human exposures.[1] The town was also environmentally contaminated with polychlorinated biphenyls (PCBs), including dioxin-like co-planar PCBs (C-PCBs), and the DDT metabolite *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE).[2] The primary aim of the Russian Children's Study (RCS) was to examine the association of these organochlorine compounds (OCs) with somatic growth, sexual maturation and semen quality. In the present report, we examined whether serum concentrations of OCs were associated with height and body mass index (BMI; kg/m^2) from age 8 to 18 years.

Materials and Methods

Study Population: The RCS is a prospective cohort study of 516 peripubertal boys, enrolled at ages 8 and 9 years from 2003 to 2005. The boys' initial study visit included physical examinations, fasting blood samples for OCs, and completion of health, dietary, lifestyle and SES questionnaires. For this analysis, 17 orphans and 10 boys with chronic health conditions that affect growth were excluded. Of the remaining 489 subjects, 473 (97%) had serum DLC and PCB concentrations measured, and 350 (72%) had serum OC pesticides measured.

Sample Collection and Analysis: Sera from enrollment blood samples were stored at -35°C until shipment for analysis at the National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA for 7 dibenzo-*p*-dioxins (dioxins), 10 dibenzofurans (furans), 4 C-PCBs, 6 mono-*ortho* substituted PCBs (M-PCBs), 31 non-dioxin-like PCBs (NDL-PCBs), HCB, βHCH, and *p,p'*-DDE. The OC samples, including method blanks and two quality control samples, were spiked with a mixture of $^{13}C_{12}$-labeled dioxins, furans, PCBs, and OC pesticides as internal standards, extracted by solid phase extraction, followed by a multi-column automated cleanup and enrichment procedure,[3] and quantified using selective ion monitoring high resolution mass spectrometry.[4] Total lipids were estimated by the Phillips equation from enzymatic measurements of total cholesterol and triglycerides.[5] Concentrations below the limit of detection (LOD) were assigned a value of $LOD/\sqrt{2}$.

Statistical Methods: The exposures were the sum of lipid-standardized dioxin-like compounds (∑DLCs: dioxins, furans, C-PCBs) and the sum of their associated toxic-equivalency factor (TEF)-weighted dioxin-like concentrations, including M-PCBs (∑TEQs)[6], the sum of ∑NDL-PCBs (including M-PCBs because of their low

TEF), HCB, βHCH, and *p,p′*-DDE. These OC groups were divided into quartiles for analyses. Mixed effects linear regression models were used to evaluate the associations of higher serum OCs at age 8-9 years with age-adjusted World Health Organization z-scores for height (HT-*Z*) and BMI (BMI-*Z*) measured over ten years of follow-up (through age 18 years). An autoregressive covariance structure was utilized to account for within-boy correlation in growth measures over time. The final models for HT-*Z* and BMI-*Z* included age, birth weight, and low parental education; in addition the model for HT-*Z* included low household income whereas the model for BMI-*Z* included no biological father in the home.

Results and Discussion

***Demographics*:** Of the 473 boys included in our analysis, 63% were 8 years old at enrollment, and baseline nutrition was within appropriate guidelines.[7] 91% of the parents had higher than secondary education, 39% of households had incomes in the highest category, and 66% of the biological fathers lived in the home.

***Serum Organochlorine Concentrations*:** At age 8-9, serum OC concentrations demonstrated a wide range (Table 1). Overall, the serum OC concentrations in the RCS were much higher than those observed in the 2003-2004 US National Health and Nutrition Examination Survey (NHANES).[8] In the RCS, the 90th percentile of total TEQ and the median ∑NDL-PCB concentrations compared to concentrations in NHANES (12.1 pg TEQ/g lipid and 51.2 ng/g lipid, respectively) among 12-29 year olds were 4- to 5-fold higher. The median serum *p,p′*-DDE and HCB concentrations were approximately 3- and 12-fold higher than those in NHANES (93.6 and 13.4 ng/g lipid, respectively) among 12-19 year olds.[8] The 10th percentile of serum βHCH in the RCS was approximately 10-fold higher than the 95th percentile (8.8 ng/g lipid) among 12-19 year olds in NHANES.[8] Correlations between serum OCs ranged from high for ∑TEQs, ∑NDL-PCBs, ∑DLCs, and ∑TEQs, and ∑NDL-PCBs with βHCH (0.71-0.82) and moderate for ∑DLCs with OC pesticides and with each other (0.49-0.61).

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Table 1. Distribution of serum organochlorines among 8-9 year old Russian boys

		Percentiles				
Organochlorine Compounds	N	10th	25th	Median	75th	90th
Total dioxin-like compounds[a], (pg/g lipid)	473	224	279	362	495	670
Total 2005 toxic equivalencies[a], (pg TEQ/ g lipid)	468	9.9	14.4	21.1	33.2	51.8
Total nondioxin-like polychlorinated biphenyls[a], (ng/g lipid)	468	128	164	250	394	613
Hexachlorobenzene[b], (ng/ g lipid)	350	80	107	159	247	365
β-hexachlorocyclohexane[b], (ng/g lipid)	350	82	114	168	272	421
p,p´-dichlorodiphenyldichloroethylene[b], (ng/g lipid)	350	122	189	287	492	866

[a]Samples below the LOD (limit of detection) were assigned a value = LOD/√2; [b]None below the LOD.

***Physical Characteristics*:** At study entry, the mean (SD) HT-*Z* and BMI-*Z* were 0.12 (1.00) and -0.19 (1.26), and height and BMI were 130.1 (6.2) cm and 15.9 (2.3) kg/m^2, respectively. At age 18 years, the overall mean (SD) HT-*Z* and BMI-*Z* among the 299 young men with study visits were 0.13 (0.92) and -0.34 (1.23), and height and BMI were 177.2 (6.8) cm and 21.4 (3.8) kg/m^2, respectively. At age 18 years, 14% of boys were overweight (>1 SD above the mean BMI-*Z*)[9], and 97% had attained sexual maturity (TV≥20 mL)[10].

Associations of Serum OCs with Longitudinal Growth

Associations of Serum ΣDLCs, ΣTEQs, and ΣNDL-PCBs with HT-Z and BMI-Z

In multivariable analyses, higher quartiles of ΣNDL-PCBs compared to the lowest were associated with lower HT-*Z*, approximating a plateau (Table 2). Higher ΣTEQs and ΣDLCs were associated with lower HT-*Z*; quartiles 2 and 3 for ΣTEQs compared to quartile 1 were significantly associated with lower HT-*Z*, as was quartile 3 for ΣDLCs, but for both the associations with the highest quartiles (4) were attenuated.

In multivariable analyses, higher quartiles of ΣTEQs, ΣDLCs, and ΣNDL-PCBs were significantly associated with lower BMI-*Z* (Table 2). The associations for ΣNDL-PCBs and ΣDLCs plateaued at quartiles 3 and 4 compared to quartile 1. For ΣTEQs, the dose-response was nonlinear, with quartiles 2-4 compared to 1 associated with lower BMI-*Z*, but with some attenuation for quartile 4.

Table 2. Associations of serum dioxin-like compounds (DLCs), toxic equivalents (TEQs), and nondioxin-like polychlorinated biphenyls (NDL-PCBs) with growth in the Russian Children's Study over ten years (N=473)

	TEQ Quartiles[a] (pg/g lipid)		DLC Quartiles[b] (pg/g lipid)		NDL-PCB Quartiles[c] (ng/g lipid)	
Exposure	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value
	Annual WHO age-adjusted height (HT) z-scores[d]					
Quartile 1	Reference		Reference		Reference	
Quartile 2	-0.26 (-0.48, -0.03)	0.02	-0.22 (-0.44, 0.007)	0.06	-0.36 (-0.58, -0.13)	0.002
Quartile 3	-0.37 (-0.59, -0.14)	0.002	-0.39 (-0.62, -0.17)	<0.001	-0.37 (-0.59, -0.14)	0.001
Quartile 4	-0.18 (-0.40, 0.05)	0.12	-0.06 (-0.29, 0.16)	0.59	-0.32 (-0.54, -0.09)	0.006
Trend test		0.08		0.31		0.008
	TEQ Quartiles[a]		DLC Quartiles[b]		NDL-PCB Quartiles[c]	
	Annual WHO age-adjusted body mass index (BMI) z-scores[e]					
Quartile 1	Reference		Reference		Reference	
Quartile 2	-0.58 (-0.86, -0.30)	<0.001	-0.50 (-0.78, -0.23)	0.005	-0.60 (-0.87, -0.33)	<0.001
Quartile 3	-0.86 (-1.14, -0.58)	<0.001	-0.70 (-0.97, -0.42)	<0.001	-0.92 (-1.20, -0.65)	<0.001
Quartile 4	-0.73 (-1.01, -0.45)	<0.001	-0.69 (-0.97, -0.41)	<0.001	-0.94 (-1.21, -0.67)	<0.001
Trend test		<0.001		<0.001		<0.001

[a]TEQ quartiles: Q1 4.0 – 14.3; Q2 14.4 – 21.1; Q3 22.0 – 33.2; Q4 33.3 – 174.7; [b]DLC quartiles: Q1 123 – 278; Q2 279 – 361; Q3 362 – 494; Q4 495 –2963; [c]NDL-PCB quartiles: Q1 62 – 163; Q2 164 -249; Q3 250 – 393; Q4 394 - 4248; [d]Adjusted repeated measures linear regression model, with birthweight, low household income, low parental education, and age (yrs).[e]Adjusted repeated measures linear regression model, with birthweight, no biological father in home, low parental education, and age (yrs).

Associations of Serum OC Pesticides with HT-Z and BMI-Z

In multivariable models, increasing quartiles of *p,p´*-DDE were associated with lower HT-*Z* (Table 3). Neither βHCH nor HCB were associated with HT-*Z*.

In multivariable models, higher quartiles of *p,p´*-DDE and βHCH were associated with lower BMI-*Z* (Table 3). Higher quartiles of HCB were associated with lower BMI-*Z* in adjusted models, with quartile 3 compared to 1 having the greatest decline but with some attenuation for quartile 4 compared to 1.

Table 3. Serum OC pesticides (ng/g lipid) associations with measures of growth in the Russian Children's Study over ten years (N=350)

	p,p´-DDE Quartiles[a]		βHCH Quartiles[b]		HCB Quartiles[c]	
Exposure	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value
	Annual WHO age-adjusted height (HT) z-scores[d]					
Quartile 1	Reference		Reference		Reference	
Quartile 2	-0.11 (-0.37, 0.15)	0.40	0.03 (-0.23, 0.29)	0.82	-0.14 (-0.41, 0.12)	0.28
Quartile 3	-0.24 (-0.50, 0.02)	0.07	-0.18 (-0.43, 0.08)	0.19	-0.06 (-0.32, 0.20)	0.64
Quartile 4	-0.48 (-0.74, -0.22)	<0.001	-0.17 (-0.44, 0.09)	0.19	-0.12 (-0.39, 0.14)	0.36
Trend test		<0.001		0.08		0.50
	Annual WHO age-adjusted body mass index (BMI) z-scores[e]					
Quartile 1	Reference		Reference		Reference	
Quartile 2	-0.70 (-1.03, -0.38)	<0.001	-0.63 (-0.94, -0.31)	<0.001	-0.44 (-0.76, -0.12)	0.008
Quartile 3	-0.81 (-1.13, -0.49)	<0.001	-1.13 (-1.44, -0.82)	<0.001	-1.07 (-1.39, -0.75)	<0.001
Quartile 4	-1.19 (-1.52, -0.86)	<0.001	-1.25 (-1.56, -0.93)	<0.001	-0.83 (-1.15, -0.50)	<0.001
Trend test		<0.001		<0.001		<0.001

[a]*p,p´*-DDE quartiles: Q1 48 – 188; Q2 189 – 286; Q3 287 – 493; Q4 494 – 9370; [b]βHCH quartiles: Q1 39 – 113; Q2 114 – 167; Q3 168 – 272; Q4 273 –2860; [c]HCB quartiles: Q1 31 – 106; Q2 107 -158; Q3 159 – 247; Q4 248 - 2660; [d]Adjusted repeated measures linear regression model, with birthweight, low household income, low parental education, and age (yrs). [e]Adjusted repeated measures linear regression model, with birthweight, no biological father in home, low parental education, and age (yrs)

Conclusions

In a longitudinal study of Russian boys assessed from the peripubertal period to young adulthood, we found that higher serum ΣNDL-PCBs and *p,p´*-DDE measured at age 8 to 9 years old were associated with lower HT-*Z* over 10 years of follow-up to age 18 years. We found that higher serum concentrations of all OCs were associated with lower BMI-*Z*. In future analyses, we will utilize bio-electric impedance measurements to assess whether higher OCs were associated with changes in body composition, and explore joint contributions of multiple OC exposures.

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