HHS Public Access

Author manuscript

Environ Res. Author manuscript; available in PMC 2023 September 01.

Published in final edited form as:

Environ Res. 2022 September ; 212(Pt A): 113218. doi:10.1016/j.envres.2022.113218.

Associations of prepubertal urinary phthalate metabolite concentrations with pubertal onset among a longitudinal cohort of boys

Jane S Burns[a], **Oleg Sergeyev**[b,c], **Mary M. Lee**[d], **Paige L. Williams**[e,f], **Lidia Mínguez-Alarcón**[a,g], **Bora Plaku-Alakbarova**[a,h], **Sergey Sokolov**[i], **Sergey Kovalev**[i], **Holger M. Koch**[j], **Albert T. Lebedev**[i], **Russ Hauser**[a,f], **Susan A. Korrick**[a,g],

Russian Children's Study

[a]Environmental and Occupational Medicine and Epidemiology Program, Department of Environmental Health, Harvard T.H. Chan School of Public Health, 665 Huntington Avenue, Building 1, Boston, MA, 02115, USA

[b]Group of Epigenetic Epidemiology, Belozersky Research Institute Of Physico-Chemical Biology, Lomonosov Moscow State University, Leninskye Gory, House 1, Building 40, room 322, 119992, Moscow, Russia

[c]Chapaevsk Medical Association, Meditsinskaya Str., 3a, Chapaevsk, Samara Region, 446100

[d]Nemours Children's Health, 1600 Rockland Road, Wilmington, 19803 and Department of Pediatrics, Sidney Kimmel Medical School, Jefferson University Philadelphia, PA USA.

[e]Department of Biostatistics, Harvard T.H. Chan School of Public Health, 665 Huntington Avenue, Building 2, Room 443, Boston, MA, 02115, USA

[f]Department of Epidemiology, Harvard T.H. Chan School of Public Health, 677 Huntington Ave., Kresge Building, 9th floor, Boston, MA, 02115, USA

[g]Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, 401 Park Drive, 3rd floor West, Boston, MA, 02215, USA

[h]Epidemiology Division, Optuminsight Life Sciences, Boston, MA, USA

Corresponding author: Jane S Burns, Environmental and Occupational Medicine and Epidemiology Program, Harvard T.H. Chan School of Public Health, 665 Huntington Avenue, Building 1, Room 1404C, Boston, MA 02115 USA. Telephone: 617-432-1829. jburns@hsph.harvard.edu.

The study was approved by the Human Studies Institutional Review Boards of the Chapaevsk Medical Association, Harvard T.H. Chan School of Public Health, Nemours Children's Health, and Brigham and Women's Hospital. Before participation, the parent/guardian provided informed consent and the boys signed assent forms. At ages 18 and above, each boy signed informed consent.

Disclaimers: The opinions in this article are those of the authors and do not necessarily reflect the official opinion of the U.S. EPA or NIEHS.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

[i]Chemistry Department, Lomonosov Moscow State University, Moscow 119991, Leninskie Gory 1/3, Russian Federation

[j]Institute for Prevention and Occupational Medicine of the German Social Accident Insurance – Institute of the Ruhr University Bochum (IPA), Bürkle-de-la-Camp-Platz 1, 44789 Bochum, Germany

Abstract

Background: Although phthalate exposures have been associated with adverse effects on male reproductive health, few studies have explored longitudinal associations with male pubertal development.

Objectives: We examined the association of prepubertal urinary concentrations of phthalate metabolites with age at pubertal onset in a prospective cohort of Russian boys.

Methods: At enrollment at ages 8–9 years, medical history, dietary, and demographic information was collected. At entry and annually, physical examinations and pubertal staging [Genitalia (G), Pubarche (P), and testicular volume (TV, in ml)] were conducted and spot urines were collected. Prepubertal urine samples (defined as either TV=1, 2 and G=1, 2 or TV=3 and G=1) were pooled for each boy and phthalate metabolite concentrations were quantified using isotope dilution LC-MS/MS at Moscow State University. We measured 15 metabolites including those from anti-androgenic parent phthalates (AAPs) such as di(2-ethylhexyl) (DEHP) and di-isononyl (DiNP) phthalates as well as monobenzyl (MBzP), mono-n-butyl (MnBP), and mono-isobutyl (MiBP) metabolites. We calculated the molar sums of DEHP (∑DEHP), DiNP (∑DiNP), and AAP (∑AAP) metabolites. Separate interval-censored models were used to assess associations of quartiles of prepubertal phthalate metabolites with each pubertal onset indicator, G2+, P2+ and TV >3mL, adjusted for covariates and urine specific gravity.

Results: 304 boys had 752 prepubertal urine samples (median 2, range: 1–6) for pooling. In adjusted models, higher urinary AAPs were consistently associated with later pubertal onset (P2) with mean shifts ranging from 8.4–14.2 months for the highest versus lowest quartiles. Significantly later onset for G2 and TV>3ml was observed for higher versus lower quartiles of MiBP, MBzP, ∑DEHP and ∑DiNP.

Conclusions: On average, boys with higher concentrations of prepubertal urinary AAPs had later pubertal onset by six months to over a year. The impact of AAPs on timing of male puberty may be attributable to disruption of androgen-dependent biological pathways.

Keywords

phthalates; endocrine disrupting chemicals; environment; children; puberty; Tanner staging

1. Introduction

Phthalates are a class of synthetic chemicals considered potential human endocrine disruptors based, in part, on the association of some phthalates with adverse male reproductive development in rodents (Howdeshell et al. 2017; Hoyer et al. 2018). High molecular weight phthalates (HMWPs), e.g. di-2-ethylhexyl (DEHP), di-isononyl (DiNP),

and di-isodecyl (DiDP), have been primarily used as plasticizers in polyvinyl chloride plastic products, ranging from plastic flooring, rainwear and shoes, children's toys, food packaging, and medical devices. Low molecular weight phthalates (LMWPs), e.g. diethyl (DEP), di-n-butyl (DnBP), and di-isobutyl (DiBP), are used as solvents in personal care products such as shampoos, lotions, cosmetics, and insect repellant (Duty et al. 2005). Exposure is pervasive in many countries and widely detected in the environment (Heudorf et al. 2007; Lebedev et al. 2018; Mazur et al. 2021; Polyakova et al. 2018; Wormuth et al. 2006). Human exposure is primarily by ingestion for HMWPs, and via [1]indoor air and dermal contact for LMWPs (Koch et al. 2013; Wormuth et al. 2006). Although these chemicals have short biological half-lives of hours (Koch and Calafat 2009), due to their widespread use there is ubiquitous and concurrent human exposure to multiple phthalates. Regulations have restricted the use of some phthalates, and they have been replaced by other, less well characterized phthalates, e.g. DiNP and DiDP for DEHP, or non-phthalate alternatives (Koch et al. 2017; Zota et al. 2014).

In rodent studies, gestational exposure to several anti-androgenic phthalates (AAPs), e.g. DEHP and DiBP, have been associated with decreased fetal testicular testosterone production and testis weight, and male reproductive tract anomalies (Hannas et al. 2011; Kay et al. 2014). Moreover, in animal studies, exposure to AAPs during gestational and juvenile periods were associated with delayed puberty and sexual maturity, and impaired semen quality (Howdeshell et al. 2017; Kay et al. 2014). In epidemiological studies, prenatal exposure to AAPs have been associated with male reproductive tract anomalies, and among men higher cross-sectional urinary AAPs were associated with reduced serum testosterone and impaired semen quality (Kay et al. 2014; Marsee et al. 2006; Radke et al. 2018), leading to the recognition of AAPs as human reproductive toxicants. However, despite evidence of male reproductive toxicity and the central role of pubertal development in reproductive health, there are few epidemiologic studies that have examined the associations between urinary concentrations of AAP metabolites with the timing of male puberty and their findings have been inconsistent.

The Russian Children's Study (RCS) is a unique and well-characterized longitudinal cohort of boys enrolled at ages 8–9 years and followed through adolescence with annual physical examinations including pubertal staging by the same physician. During the annual study visits, spot urine samples were collected and archived, and extensive data collected on early life, dietary, and demographic characteristics. In prior publications, we examined the

[1]Abbreviations: AAP, anti-androgenic phthalate; DEHP, di(2-ethylhexyl) phthalate; DEP, diethyl phthalate; DHEAS, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; DiBP, di-isobutyl phthalate; DiDP, di-isodecyl phthalate; DiNP, di-isononyl phthalate; DnBP, di-n-butyl phthalate; EDC, endocrine disrupting chemicals; G, Tanner stage genitalia; G2, Tanner stage pubertal onset by genitalia development; GM, geometric mean; HMWP, high molecular weight phthalate; LC-MS/MS, liquid chromatography tandem mass spectrometry; LMWP, low molecular weight phthalate; MBzP, mono-n-butyl phthalate; MCNP, mono-(carboxy-iso-nonyl) phthalate; MCOP, mono-carboxyl-iso-octyl phthalate; MCPP, mono-(3-carboxypropyl) phthalate; MECPP, mono(2-ethyl-5-carboxy-pentyl) phthalate; MEHHP, mono(2-ethyl-5-hydroxy-hexyl) phthalate; MEHP, mono(2-ethylhexyl) phthalate; MEOHP, mono(2-ethyl-5-oxo-hexyl) phthalate; MEP, mono-ethyl phthalate; MHiDP, mono-(hydroxy-iso-decyl) phthalate; MHiNP, mono-hydroxy-iso-nonyl phthalate; MiBP, mono-isobutyl phthalate; MnBP, mono-n-butyl phthalate; MOiDP, mono-(oxo-iso-decyl) phthalate; MOiNP, mono-oxo-iso-nonyl phthalate; P, Tanner stage pubic hair; P2, Tanner stage pubertal onset by pubic hair development; RCS, Russian Children's Study; R*s*, Spearman correlation; RSD%, inter-assay relative standard deviation percent; SES, socioeconomic status; SG, specific gravity; S/N, signal-to-noise ratio; ∑AAP, molar sum of all anti-androgenic phthalate metabolites (μmol/L); ∑DEHP, molar sum of DEHP metabolites (μmol/L); ∑DiDP, molar sum of DiDP metabolites (μmol/L); ∑DiNP, molar sum of DiNP metabolites (μmol/L); TV, testicular volume.

associations of organochlorine chemicals and lead with pubertal timing (Burns et al. 2016; Lam et al. 2014; Williams et al. 2010). We expand these observations in the current analysis by focusing on the relationship between prepubertal urinary AAP metabolite concentrations and pubertal onset.

2. Materials and Methods

2.1. Study Population

The Russian Children's Study is a prospective cohort study of 516 boys who were enrolled at ages 8–9 years from 2003 to 2005 in Chapaevsk, Russia (Hauser et al. 2005), and followed annually to ages 18–19 years as described previously (Burns et al. 2020; Sergeyev et al. 2017). The study was designed to prospectively assess the potential impact of prevalent environmental contaminants (e.g., organochlorines and metals and, most recently, phthalates) on male growth and pubertal development. The study was approved by the Human Studies Institutional Review Boards of the Chapaevsk Medical Association, Harvard T.H. Chan School of Public Health, Nemours Children's Health, and Brigham and Women's Hospital. Before participation, the parent/guardian provided informed consent and the boys signed assent forms. At age 18 and above, each boy signed informed consent. Among boys with urinary phthalate metabolites measured at any visit (506/516, 98%), 320 (63%) had concentrations measured prior to pubertal onset. Exclusions included boys who were orphans with missing birth and parental information (n=13) and those with chronic diseases that could affect puberty (n=3), with the final sample size of 304.

At study entry, each boy's parent or guardian completed nurse-administered health and lifestyle questionnaires on early childhood and medical history, and demographic and socioeconomic status (SES) (Lee et al. 2003). A validated Russian Institute of Nutrition semi-quantitative food frequency questionnaire was completed by the parent/guardian for each boy (Martinchik et al. 1998; Rockett and Colditz 1997). Birth information was abstracted from medical records.

2.2. Physical Examination and Pubertal Assessment

At study entry, a standardized anthropometric examination and pubertal staging was performed by one physician (O.S.). Annual follow-up physical examinations were performed by a single trained research nurse and pubertal staging by O.S. without knowledge of urinary phthalate metabolites concentrations. Staging of genitalia (G) and pubic hair (P) as 1 (immature) to 5 (sexually mature) was by visual inspection (Tanner and Whitehouse 1976). Testicular volume (TV) was measured using a Prader orchidometer. Prepuberty was defined as either TV=1, 2 and G=1, 2 or TV=3 and G=1. Pubertal onset was defined as G2, P2, or TV>3 mL for either testis.

2.3. Urinary Phthalate Metabolite Assessment

At enrollment and annually, spot urines collected in clean polypropylene containers were aliquoted into 15 ml sterile glass containers and stored at −35^{o}C.

For each boy, an aliquot of urine from each annual exam was defrosted, vortexed, and pooled into one of four pubertal categories (prepuberty, early puberty, mid to late puberty, or sexual maturity) using pre-specified G and TV criteria. The 304 boys had 752 urine samples available for the prepubertal period; each boy had 1 to 6 samples (median=2, IQR 1–3) to contribute to his individual pooled sample. Pools for each boy were made by combining individual annual prepubertal aliquots. The pooled urine was thoroughly vortexed before measuring specific gravity (sg) and aliquoting into 1.8 ml polypropylene cryovials for storage at −35°C. Urine samples collected during the first ten months of enrollment, n=216, were stored at the Harvard T.H. Chan School of Public Health in Boston, Massachusetts, U.S.A. and unavailable for pooling because of Russian restrictions on shipping specimens to the Moscow State University (MSU) laboratory, Moscow, Russia.

The frozen pooled urine samples were transported in dry ice from Chapaevsk to MSU for analysis according to the methods of Koch et al. (Koch et al. 2017). Phthalate metabolite concentrations were measured using online liquid chromatography tandem mass spectrometry (LC-MS/MS) (Koch et al. 2017). Urinary phthalate metabolites measured were mono-ethyl phthalate (MEP), mono-isobutyl phthalate (MiBP), mono-benzyl phthalate (MBzP), mono-n-butyl phthalate (MnBP), mono(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-hydroxy-hexyl) phthalate (MEHHP), mono(2-ethyl-5-oxo-hexyl) phthalate (MEOHP), mono(2-ethyl-5-carboxy-pentyl) phthalate (MECPP), mono-hydroxy-iso-nonyl phthalate (MHiNP), mono-oxo-iso-nonyl phthalate (MOiNP), mono-carboxy-iso-octyl phthalate (MCOP), mono-(hydroxy-iso-decyl) phthalate (MHiDP), mono-(oxo-iso-decyl) phthalate (MOiDP), mono-(carboxy-iso-nonyl) phthalate (MCNP), and mono-(3-carboxypropyl) phthalate (MCPP) (see Table 1 for parent phthalates and their metabolites). Calibrations were performed using commercial reference standards from LGC (MEP, MiBP, MnBP, MBzP, MEHP; Teddington, UK), Biozol (MEHHP, MEOHP, MECPP; Eching, Germany), custom synthesized standards provided by Koch/IPA (MCPP, MHiNP, MOiNP, MCOP, MHiDP, MOiDP, MCNP; Bochum, Germany) and isotopically labelled internal standards from Toronto Research Chemicals (MEP; Toronto, Canada), LGC (MCPP, MEHP, MEOHP; Teddington, UK) and custom synthesized by Koch/IPA (MiBP, MnBP, MBzP, MEHHP, MECPP, MHiNP, MOiNP, MCOP, MHiDP, MOiDP, MCNP; Bochum, Germany). Analyses were performed in 34 batches of 50 samples each including two randomly selected participants' samples analyzed in duplicate, two quality control (QC) samples (from designated discard urines) with known low and high concentrations of each metabolite (1 QC_{low} and 1 QC_{high}), and 1 field blank. For each boy, pooled urines from each pubertal stage were analyzed in the same batch. When a peak was absent or indeterminate, zero was assigned. Limits of detection (LOD) were defined as a signal-to-noise (S/N) ratio of 3 in a urine matrix assessed as part of method validation. The inter-assay relative SD percent (RSD%: (SD/mean)*100) across the 34 batches for QC_{high} ranged from 1.5–20.0%, except for MEHP (29.1%), whereas RSD% for QC_{low} were ≤20%, except for MEHP (50.8%) and DiDP metabolites (23.6 – 57.8%), one of which had 23% of values below the LOD.

2.4. Statistical Analysis

Prepubertal urinary metabolite concentrations of AAPs were the *a priori* primary focus of our analyses; MBzP (ng/mL), MiBP (ng/mL), MnBP (ng/mL), the molar sum of DEHP

metabolites (∑DEHP, μmol/L), the molar sum of DiNP metabolites (∑DiNP, μmol/L), and the molar sum of all metabolites of the five AAPs, with DiNP metabolites weighted 0.43 because of its weaker relative impact on fetal testosterone production (Hannas et al. 2011), (∑AAP, μmol/L). In addition, we performed analyses examining the associations between prepubertal metabolites of phthalates without recognized anti-androgenic activity: MEP (ng/mL), the molar sum of DiDP metabolites (∑DiDP, μmol/L), and MCPP (ng/mL). Of note, MCPP is a metabolite of multiple parent phthalates, both HMW and LMW phthalates, e.g. DnBP, DiNP, DiDP, thus representing potential exposures to both AAPs and non-AAPs. Finally, for completeness we assessed the associations of individual metabolites of DEHP (i.e., MEHP, MEHHP, MEOHP, and MECPP) and DiNP (i.e., MHiNP, MOiNP, and MCOP) with age at pubertal onset.

Unadjusted and adjusted interval-censored survival analyses were used to evaluate the associations of boys' prepubertal urinary phthalate metabolite concentrations with age at pubertal onset. We used quartiles of urinary phthalate metabolites with the lowest quartile as the reference. This conservative approach does not require an assumption of linearity and provides estimates of differences in pubertal onset in months associated with different quartiles of prepubertal urinary phthalate metabolites. Age at pubertal onset was 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 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). Using this interval-censored model, we estimated the overall mean age of onset for each pubertal onset indicator. Parameter estimates using maximum likelihood methods were obtained via PROC LIFEREG in SAS Version 9.2 (SAS Institute, Cary NC).

A separate model was fit for each measure of pubertal onset; all models were adjusted for urine specific gravity. All covariates except height and body mass index (BMI, kg/m^2) were identified as *a priori* predictors of pubertal development and were considered for inclusion in the models (Table 2). A core model was developed by first evaluating associations of each covariate with pubertal onset and retaining those with $p<0.20$, then including these in a full model and using backwards selection to exclude covariates with $p>0.10$. To check for confounding, excluded covariates with $p<0.20$ were added individually to the final model and those associated with ≥ 10% change in phthalate trend test coefficients retained. Statistical significance was defined as $p\leq0.05$. Missing covariate data was minimal and thus a complete case analysis was conducted.

By definition, *prepubertal* urinary pools included some boys who were in early puberty (G2 or P2) based on discordant sexual maturity measures. Because of this, sensitivity analyses, excluding boys who were in G2 or P2 in their prepubertal urine pool, were conducted to assess robustness of associations between urinary phthalate metabolites and pubertal onset, reducing the sample number to 228. Growth is closely linked with pubertal onset; therefore, an additional sensitivity analysis was conducted adjusting for the boys' height and BMI at age 9 years.

Because over 25% of the pooled samples consisted of one urine sample, we assessed differences in distribution and concentrations by number of urines in the pooled samples (categories: 1 (n=99), 2 (n=67), 3 (n=64), and 4+ (n=74)) across quartiles of phthalate metabolite concentrations, using Mantel-Haenszel chi-square and Kruskal Wallis statistical tests, respectively. When significant differences were detected, we performed sensitivity analyses restricting the analysis to 205 boys who had 2+ urine samples in their pooled sample.

3. Results

3.1. Study population

Table 2 summarizes study participants' perinatal history, and baseline anthropometric measurements, diet, maternal and household characteristics, and missing data, which was minimal. At study entry (age 8–9 years), the majority of boys were within the normal range for height and BMI, with 13% overweight (de Onis et al. 2007). Half of the boys had prenatal exposure to household and/or maternal tobacco smoke, and 13% of mothers reported alcohol consumption during pregnancy. Although over 90% had at least one parent with more than a secondary education, a third of the families were in the lowest household income level at the time of study entry. The boys' dietary intake of total calories and macronutrients were within recommended levels for their age group and sex (Food and Nutrition Board 2006). Among the 304 boys included in this analysis, at study entry (age 8 to 9 years), pubertal onset had occurred in 7% (P2), 8% (G2), and none using TV>3 mL. The estimated mean (95% CI) age of pubertal onset by P2, G2, and TV>3mL was 12.1 (11.9, 12.3), 10.3 (10.1, 10.5), and 11.1 (11.0, 11.3) years, respectively.

3.2. Urinary phthalate metabolites

The 304 boys who contributed to the prepubertal urine pools were 8 to 13 (median 9) years of age at sample collection, which spanned 2004–2009 (median 2005). Most of the phthalate metabolite measurements were >LOD, except for the DiDP metabolite MOiDP (23% <LOD) (Table 3). The range of metabolite concentrations was wide, with the 90th percentiles for MnBP, MEHHP, MECPP, and MEP being over 200 ng/mL. Concentrations of DiDP metabolites were lower than most other metabolites, with medians below 1.0 ng/mL for MOiDP and MCNP. The metabolite MBzP and DiNP metabolites MHiNP, MOiNP, and MCOP were also at lower concentrations than other metabolites, with medians <10 ng/mL. Most of the phthalate metabolites were moderately positively correlated [Spearman r (r_s) 0.30 to 0.64], with higher correlations among the metabolites of DEHP (r_s 0.71 to 0.96) and DiNP (r_s 0.88 to 0.92). Phthalate metabolites were weakly correlated with organochlorine chemicals (sum of dioxin-like toxic equivalents, sum of nondioxin-like polychlorinated biphenyls, *p,p′*-dichlorodiphenyldichloroethylene, hexachlorobenzene, and beta-hexachlorocyclohexane) (r_s −0.09 to 0.26) and blood lead (r_s −0.18 to 0.17) measured at study enrollment.

Comparing biomarkers of phthalate exposure in the RCS with similar aged cohorts elsewhere in Europe and the U.S., there were regional and cohort-specific variability in LMWP and HMWP metabolite concentrations (Table S1). Notably, urinary concentrations

of LMWP MEP and MnBP and HMWP DEHP metabolites in the Russian cohort were substantially higher than in European and U.S. cohorts (CDC 2019; Gari et al. 2019; Kasper-Sonnenberg et al. 2014). Urinary concentrations of MBzP in RCS were much lower than in U.S. cohorts (CDC 2019), but similar to European cohorts (Gari et al. 2019; Kasper-Sonnenberg et al. 2014).

3.3. Prepubertal phthalates and pubertal onset

Overall, the highest compared to the lowest levels of the urinary prepubertal metabolites of any AAPs were associated with later onset of pubertal pubic hair development (pubarche), with mean shifts ranging from approximately 8 to 14 months (Table 4). For MnBP, only Q4 versus Q1 was associated with significantly later P2 of 9.3 (95% CI 1.5, 17.1) months. The dose-response relationship of quartiles of MiBP with P2 appeared linear, whereas the associations of MBzP and ∑DEHP with P2 were somewhat attenuated at Q4. The dose-response association between quartiles of ∑DiNP and P2 demonstrated a plateau at Q3-Q4 compared to Q1 of approximately 8.6 months. The dose-response relationship over quartiles of ∑AAP with P2 appeared to be linear (trend p=0.006).

The associations between urinary metabolites of prepubertal AAPs and pubertal onset by TV and G did not demonstrate a consistent pattern (Table 4). MnBP was not associated with age at pubertal onset by G nor TV. Quartiles 2 and 3 versus quartile 1 of MiBP were associated with later pubertal onset by G and TV, but the associations for quartile 4 were attenuated. For MBzP, the highest versus lowest quartiles were associated with 7.5 (95% CI 1.1, 13.8) months later G2 (trend p-value=0.02) and 5.6 months (95% CI 0.6, 10.7) later TV onset (trend p-value=0.006). Quartiles 3 versus 1 of ∑DEHP were significantly associated with later pubertal onset by G and TV, 8.0 (2.9, 13.2) and 8.3 (1.8, 14.7) months, respectively, but there were no clear associations with Q4. ∑DiNP metabolites were associated with approximately 6 months (95% CI) later G2 for Q3 (0.03, 13.0) and Q4 (−0.6, 12.7) versus Q1 and 5.4 (0.01, 10.7) months later TV onset for Q4 versus Q1. ∑AAP were not significantly associated with TV onset, but there was a significantly later G2 associated with Q3 versus Q1 of 9.1 (2.4, 15.8) months, but no clear association with Q4.

In analyses examining the relationships between metabolites of *non*-anti-androgenic phthalates, the highest versus lowest quartile of MEP was associated with 7.1 (95% CI −0.1, 14.2) months later P2 (trend p=0.03) (Table S2). Quartile 2 versus 1 of MEP was associated with later onset by G and TV of 8.3 months (95% CI 2.2, 14.4) and 7.9 months (95% CI 3.0, 12.8), respectively, but associations with Q3 and Q4 were greatly attenuated. ∑DiDP was not associated with pubertal onset (Table S2). Quartile 4 versus 1 of MCPP was associated with 9.1 months later P2 (95% CI 1.3, 16.9); otherwise MCPP was not associated with later TV onset nor G2.

Generally, we found that the associations of individual DEHP metabolites (MEHP, MEHHP, MEOHP, and MECPP) and DiNP metabolites (MHiNP, MOiNP, and MCOP) with age at pubertal onset were similar to their sums (∑DEHP and ∑DiNP) (Table S3).

3.4. Sensitivity analyses

In analyses restricted to boys who were prepubertal for both G1 **and** P1 at the time of urine collection (n=228), associations were generally similar (Table S4). There was slight attenuation of some associations between AAP metabolites and P2, but all remained significant (Table S4). The associations between MiBP and onset by G and TV were strengthened, comparing Q4 versus Q1, from 6.2 months to 8.0 months later (95% CI 1.1, 14.9) and from 5.7 to 7.8 months (95% CI 1.6, 14.1), respectively.

In analyses that further adjusted for height and BMI at age 9 years, the associations between urinary prepubertal metabolites of AAP metabolites and P2 remained similar (Table S5). The associations of urinary MnBP and MBzP with onset by G and TV did not change after further adjustment by height and BMI. However, most of the associations between MiBP, ∑DEHP, ∑DiNP, and ∑AAP and onset by G and TV were attenuated (Table S5).

We found that MiBP, MBzP, and ∑DiNP had proportionally more 1 urine pools in the lowest quartile (41–51%) than in the highest quartile (20–26%). Additionally, a comparison of the median concentrations of these metabolites across the categories of pooled urine samples suggests the pooled urines with a higher number of samples had higher phthalate concentrations (Table S6). For MnBP, ∑DEHP, and ∑AAPs the number of samples pooled did not vary by quartile and the median phthalate concentrations did not vary by number of urine samples pooled. In this sensitivity analysis, the relationship of MiBP and ∑DiNP with all three measures of pubertal onset were substantially attenuated although pubarche associations remained (Table S7). However, associations of MBzP with pubertal onset were largely unchanged.

4. Discussion

Higher prepubertal urinary concentrations of AAP metabolites were associated with later pubarche (P2). This was evident for both individual and summed phthalate metabolites, with mean delays of 8 to 14 months for the highest versus lowest exposure quartiles. We also observed that higher prepubertal urinary concentrations of MiBP, MBzP, and ∑DiNP were associated with later gonadarche (TV>3mL, G2), but the delays in age of gonadarche were less than that observed for pubarche and ranged from 5.4 to 7.5 months. In addition, for ∑DEHP and gonadarche, there was an approximately 8 month later onset observed with Q3 versus Q1 but no appreciable difference in onset age for Q4 versus Q1.

Toxicological studies in rats have shown that pre- and post-natal AAP exposures are associated with reduction in testicular testosterone production, and altered male reproductive development and function, including delayed puberty (Howdeshell et al. 2017; Kay et al. 2014). Epidemiological data, however, are limited and inconsistent, including 4 cross-sectional cohort studies. Two studies in China among boys (ages 6–14 years) found associations of higher urinary DEHP metabolites with later pubarche, and higher MnBP was associated with smaller TV (Shi et al. 2015; Zhang et al. 2015). However, studies in Danish (n=555, ages 6–19 years) and Mexican (n=113, ages 8–14 years) male cohorts did not observe any associations between childhood urinary phthalate concentrations and Tanner staging of pubarche nor genitalia (Ferguson et al. 2014; Mieritz et al. 2012). It

is hypothesized that AAP exposures, particularly during hormonally sensitive pre- and peripubertal periods, could impact pubertal timing by interfering with androgen production (dehydroepiandrosterone (DHEA) and testosterone) and signaling in males; therefore assessing longitudinal associations that capture critical exposure windows may be more relevant than a cross-sectional analysis.

A Mexican study, Early Life Exposure in Mexico to Environmental Toxicants (ELEMENT), reported that higher versus lower maternal urinary concentrations of MBzP (an AAP metabolite) during the third trimester of pregnancy were associated with later pubarche among boys 8–14 years old, but there were no associations between phthalates and gonadarche (Ferguson et al. 2014). Additionally, boys whose mothers had higher versus lower urinary DEHP metabolites in the third trimester had lower odds (OR range 0.12–0.36) of pubarche (Ferguson et al. 2014). A U.S. study among 9–13-year-old children, the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS), where 60% of the boys were overweight, found significant modification of the associations of maternal urinary HMWPs (MCNP, MCOP, MCPP, ∑DEHP) and MBzP measured during pregnancy with pubertal onset by BMI. While among overweight (n=89) boys, higher versus lower maternal urinary HMWPs and MBzP were associated with earlier pubarche and gonadarche, among normal weight boys (n=55), the only significant association was between higher versus lower MBzP and later pubarche (Berger et al. 2018). It is important to note that unlike the ELEMENT and CHAMACOS studies, the timing of our exposure versus their exposure was different, and most of the boys in the Russian cohort were of normal weight (87%), making it difficult to assess effect modification by BMI. Neither the CHAMACOS nor the ELEMENT studies observed associations between other LMWPs, i.e. MEP, MnBP, and MiBP, and male pubertal onset (Ferguson et al. 2014; Harley et al. 2019). However, a subsequent analysis of the ELEMENT cohort that included additional follow-up, did observe an association between higher versus lower maternal urinary MnBP and lower odds of being at a more advanced stage of pubarche at ages 8–14 years (OR=0.37; 95% CI 0.14, 0.95) (Cathey et al. 2020). Overall, the findings of increasing urinary AAP metabolite concentrations and later pubarche were consistent across RCS and these two cohorts, despite differences in timing of exposure (prepubertal versus prenatal) and study participants' ethnicity.

Two epidemiological studies assessed associations between peripubertal urinary AAPs and male pubertal onset or early progression, and used approaches that averaged the urinary prepubertal and pubertal phthalates collected during follow-up (Mouritsen et al. 2013; Zhang et al. 2015), as opposed to our method that used TV and G to identify and combine *prepubertal* urine samples. A longitudinal Danish study enrolled 168 children (84 boys), ages 5–12 years, and followed them for 5 years with first morning urine collection and physical examinations every six months (Mouritsen et al. 2013). Over the five-year follow-up period, each phthalate metabolite urinary concentration (ng/ml) was determined for each child, multiplied by urine volume (ml), and divided by body weight (kg), producing an amount excreted per kilogram body weight. An individual and agroup geometric mean for each gender were calculated, with each child assigned to a high versus low exposure group, depending on whether their mean was above or below the group mean. Among boys at age 11 years, higher versus lower MBP (combined MnBP and MiBP) was associated with

lower adrenal androgens, specifically dehydroepiandrosterone sulfate (DHEAS), thereby providing a potential mechanism consistent with our findings. Paradoxically, though, higher versus lower MBP was associated with earlier male pubarche, on average occurring at 11.0 years versus 12.3 years, respectively, which is inconsistent with our findings and DHEAS results. A Chinese study assessed the associations between urinary phthalate metabolites and pubertal onset and progression over 18 months among 430 children, ages 6–13 years (Zhang et al. 2015). For 78% of the children, the average urinary phthalates measured at enrollment and at the end of follow-up were calculated, representing peripubertal exposure; for the rest of the children the single urine collected at the end of follow-up was used to estimate exposure. Among the subset of boys who were prepubertal (defined as TV=1 mL) at entry (n=118), no associations were observed between phthalate metabolites and pubarche or gonadarche. There may be several reasons for the contradictory findings comparing the Danish and Chinese cohorts to ours. In the Danish and Chinese cohorts, the estimated exposure included urine biomarker collection before and after pubertal onset, an approach that may lead to significant exposure misclassification. Additionally, urinary MnBP and DEHP concentrations ($GM_{sg\ adjusted}$ ng/mL) were much higher in the RCS cohort as compared to the Chinese cohort (MnBP 210.1 vs 27.4 and ∑MEHP, MEHHP, MEOHP 171.5 vs. 23.3, respectively) (Zhang et al. 2015).

A German study did limit urinary phthalate quantification to study enrollment at ages 8–10 years, assessing the associations between these phthalates and a pubertal development scale (PDS) that combined several indicators of pubertal development over three years of follow-up (Kasper-Sonnenberg et al. 2017). For boys, the PDS scale consisted of facial hair, voice change, and Tanner stage pubic hair. Among the 210 boys, higher versus lower DEHP metabolites were associated with advanced PDS, suggesting earlier puberty, contrary to our findings. In the subanalysis using the separate endpoints of the PDS, higher versus lower DEHP metabolites were strongly associated with earlier facial hair (OR range 2.10–3.11) in the final year of follow-up but with *later* pubarche (OR range 0.47–0.71), consistent with our findings. This suggests the association between higher DEHP metabolites and the higher overall PDS may be driven by in large part by the association with facial hair.

Notably, in our Russian cohort the association of ∑DEHP with pubertal onset for all pubertal outcomes demonstrated delays associated with Q3 versus Q1, but attenuation of associations at the highest (Q4) exposure level. For example, gonadarche occurred approximately 8 months later for Q3 versus Q1, but for Q4 was 3.6 months later for G2 and −0.1 months for TV onset. This dose-response pattern appears to be reflected in the association of ∑AAPs with gonadarche. It has been hypothesized that the effects of endocrine disrupting chemical (EDC) exposures on hormone-driven outcomes, such as puberty, may be more deleterious at lower rather than higher levels (Vandenberg et al. 2012). In our analyses of non-anti-androgenic phthalate metabolites there was a significant relationship between highest versus lowest quartiles of MEP and MCPP and later pubarche. It is difficult to interpret the association between MEP and pubarche because the parent compound of MEP, diethyl phthalate, is not anti-androgenic. Also, the urinary MEP concentrations observed in RCS were 2–3 times higher than other European and U.S. cohorts (CDC 2019; Gari et al. 2019; Kasper-Sonnenberg et al. 2014). It may be that at these high levels the association

may be the result of residual confounding. MCCP is a metabolite of several different parent phthalates, so it is difficult to ascribe an underlying mechanism to this association.

In our cohort, we found strong evidence that higher prepubertal urinary AAP metabolites were associated with later pubarche, and to a lesser extent with later gondarche. While gonadarche depends on activation of the hypothalamic-pituitary-gonadal axis, pubarche depends on adrenarche, the maturation of the innermost zone of the adrenal cortex (the zona reticularis) (Rey 2021; Witchel et al. 2020). During adrenarche the zona reticularis produces adrenal androgens, e.g. DHEA, via three pathways, i.e. the canonical, alternative "backdoor," and the 11-oxo-androgen pathways (Witchel et al. 2020). These pathways may be vulnerable to AAPs, without directly affecting testosterone production in the testes. However, since some adrenal androgens are converted to testosterone in the testicular somatic cells (Turcu et al. 2020), impairment of adrenal steroidogenesis and/or androgen signaling may also result in reduced testicular conversion of adrenal androgens to more biologically active androgens, which may in turn impact the pace of gonadarche. The apparent greater sensitivity of pubarche as compared to gonadarche to AAPs may be indicative of different biological pathways contributing to markers of puberty.

The prepubertal window is uniquely important for the initiation of pubertal onset, when sex steroid production increases, therefore we targeted this period to obtain a biomarker of average prepubertal AAP exposure. Our definition of prepubertal relied on a combination of TV and G staging that allowed some boys to contribute to their designated prepubertal urine pools who had G2 and/or P2 staging at one or more of their annual visits. In our sensitivity analysis, when we further restricted our data to those whose staging was limited to G1 and P1 at all visits included in the prepubertal urine pool, the results did not differ enough to change our overall interpretation. When our models were adjusted for height and BMI at age 9 years, we saw either no change in the estimates, or some attenuation, which may reflect the close relationship between pubertal onset and growth where growth may be an intermediate variable. These sensitivity analyses strengthen our interpretation that the associations we observed between AAPs and pubertal onset are not due to chance, misclassification of exposure, or residual confounding by growth. When we restricted our analyses to boys with at least 2 urines in the pooled samples, the relationship of MiBP and ∑DiNP with all three measures of pubertal onset were substantially attenuated although pubarche associations remained. An explanation may be that the average age of G2 and TV>3mL is 10.3 years and 11.1 years, respectively, and the sensitivity analysis eliminated boys who had pubertal onset by age 9 or 10 years, reducing the number of boys with G2 and TV>3mL, impacting our power to detect associations with these outcomes. Since the average age at P2 is 12.1 years, this would have had less impact on the associations with P2. However, sensitivity analyses suggest the potential for variability in the number of prepubertal urine samples available for pooling to have contributed to biased associations for some (MiBP and ∑DiNP), but not all, phthalate exposure biomarkers studied. The associations of phthalate biomarkers with pubertal onset by pubarche were especially robust.

The RCS is a prospective cohort that has measured numerous environmental exposures, including urinary phthalate metabolites. It is a unique strength of our study that we were able to identify and pool prepubertal urine samples among the boys in our cohort,

providing an estimate of average prepubertal phthalate exposure. To our knowledge, no other epidemiological study has done this. Another strength of our cohort is the measurement of pubertal stage by one pediatric endocrinologist (OS), using both Tanner Staging and the more objective TV measurement (Biro et al. 1995). Our cohort is well-characterized, with information collected on potential confounders, and followed annually over the course of puberty, allowing for more comprehensive characterization of pubertal development than has been possible in most studies. Moreover, since urinary phthalates were quantified separately, without knowledge of pubertal onset, information bias is unlikely.

Although several epidemiological studies have shown that prenatal phthalate exposures are associated with alterations in male puberty (Berger et al. 2018; Cathey et al. 2020; Ferguson et al. 2014), the RCS did not have prenatal maternal urine samples to estimate prenatal exposure. Therefore, we cannot assess the association of prenatal phthalate exposures with pubertal onset. That said, correlations between prenatal and childhood phthalate exposures are generally weak (Shoaff et al. 2017). Therefore, prenatal phthalate exposure is unlikely to confound our findings. Generally, urinary concentrations of phthalate metabolites in our cohort were high, especially when compared to similar populations in the U.S. and Europe (CDC 2019; Gari et al. 2019; Kasper-Sonnenberg et al. 2014), which may affect generalizability. On the other hand, phthalate exposures (especially DEHP and DnBP) might have been even higher before the turn of the millennium when rapid changes in phthalate containing products might differentially affected the different cohorts (Frederiksen et al. 2020; Koch et al. 2017; Zota et al. 2014). In the RCS we did not find that other EDCs (e.g., serum organochlorines and blood lead) were strongly correlated with urinary phthalates and therefore are unlikely to confound the observed associations; however, we did not measure other EDCs, e.g. parabens, that may be correlated with phthalates.

5. Conclusions

We found that higher versus lower AAP metabolites during the prepubertal period were strongly associated with later pubertal onset, especially pubarche. Longitudinal studies have linked clinically delayed puberty among boys to increased risk of osteopenia (Finkelstein et al. 1996) and psychosocial and behavior issues (Graber 2013). There is widespread exposure to phthalates, and our findings raise the level of concern regarding the role of anti-androgenic phthalates on male pubertal timing, with implications for adolescent and adult health. Future analyses will assess whether exposure to AAPs during the hormonally sensitive prepubertal window may also alter the levels of reproductive hormones.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments:

We would like to thank the study participants, the Chapaevsk government, and the Chapaevsk Medical Association and Chapaevsk Central Hospital staff. We also thank our colleague Boris Revich for his input in the Russian Children's Study.

Funding:

This work was funded by the U.S. Environmental Protection Agency (EPA grant R82943701) and the National Institute of Environmental Health Sciences (NIEHS grants R01 ES014370 and P30 ES000002). The funding sources played no role in the study design, the collection, analysis and interpretation of the data, or in the writing or decision to submit this manuscript for publication.

References

Berger K, Eskenazi B, Kogut K, Parra K, Lustig RH, Greenspan LC, et al. 2018. Association of prenatal urinary concentrations of phthalates and bisphenol a and pubertal timing in boys and girls. Environ Health Perspect 126:97004. [PubMed: 30203993]

Biro FM, Lucky AW, Huster GA, Morrison JA. 1995. Pubertal staging in boys. J Pediatr 127:100–102. [PubMed: 7608791]

Burns JS, Lee MM, Williams PL, Korrick SA, Sergeyev O, Lam T, et al. 2016. Associations of peripubertal serum dioxin and polychlorinated biphenyl concentrations with pubertal timing among russian boys. Environ Health Perspect 124:1801–1807. [PubMed: 27187981]

Burns JS, Williams PL, Sergeyev O, Korrick SA, Rudnev S, Plaku-Alakbarova B, et al. . 2020. Associations of peri-pubertal serum dioxins and polychlorinated biphenyls with growth and body composition among russian boys in a longitudinal cohort. Int J Hyg Environ Health 223:228–237. [PubMed: 31466867]

Cathey A, Watkins DJ, Sanchez BN, Tamayo-Ortiz M, Solano-Gonzalez M, Torres-Olascoaga L, et al. 2020. Onset and tempo of sexual maturation is differentially associated with gestational phthalate exposure between boys and girls in a mexico city birth cohort. Environ Int 136:105469.

Center for Disease Control and Prevention (CDC). (2019). Fourth National Report on Human Exposure to Environmental Chemicals, Updated Tables. Health and Human Services. One: 437–513.

Christensen KY, Maisonet M, Rubin C, Holmes A, Flanders WD, Heron J, et al. 2010. Progression through puberty in girls enrolled in a contemporary british cohort. J Adolesc Health 47:282–289. [PubMed: 20708568]

de Onis M, Onyango AW, Borghi E, Siyam A, Nishida C, Siekmann J. 2007. Development of a who growth reference for school-aged children and adolescents. Bull World Health Organ 85:660–667. [PubMed: 18026621]

Duty SM, Ackerman RM, Calafat AM, Hauser R. 2005. Personal care product use predicts urinary concentrations of some phthalate monoesters. Environ Health Perspect 113:1530–1535. [PubMed: 16263507]

Ferguson KK, Peterson KE, Lee JM, Mercado-Garcia A, Blank-Goldenberg C, Tellez-Rojo MM, et al. 2014. Prenatal and peripubertal phthalates and bisphenol a in relation to sex hormones and puberty in boys. Reprod Toxicol 47:70–76. [PubMed: 24945889]

Finkelstein JS, Klibanski A, Neer RM. 1996. A longitudinal evaluation of bone mineral density in adult men with histories of delayed puberty. J Clin Endocrinol Metab 81:1152–1155. [PubMed: 8772591]

Food and Nutrition Board, Institute of Medicine. Macronutrients and Healthful Diets. In: Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fatty Acids, Cholesterol, Protein, and Amino Acids. Washington, D.C.: National Academy Press; 2005. p. 769–879.

Frederiksen H, Nielsen OHM, Skakkebaek NE, Juul A, Jorgensen N, et al. . 2020. Changes in urinary excretion of phthalates, phthalate substitutes, bisphenols and other polychlorinated and phenolic substances in young danish men; 2009–2017. Int J Hyg Environ Health 223:93–105. [PubMed: 31669154]

Gari M, Koch HM, Palmke C, Jankowska A, Wesolowska E, Hanke W, et al. 2019. Determinants of phthalate exposure and risk assessment in children from poland. Environ Int 127:742–753. [PubMed: 31003057]

Graber JA. 2013. Pubertal timing and the development of psychopathology in adolescence and beyond. Horm Behav 64:262–269. [PubMed: 23998670]

Hannas BR, Lambright CS, Furr J, Howdeshell KL, Wilson VS, Gray LE Jr. 2011. Dose-response assessment of fetal testosterone production and gene expression levels in rat testes following in utero exposure to diethylhexyl phthalate, diisobutyl phthalate, diisoheptyl phthalate, and diisononyl phthalate. Toxicol Sci 123:206–216. [PubMed: 21633115]

Harley KG, Berger KP, Kogut K, Parra K, Lustig RH, Greenspan LC, et al. 2019. Association of phthalates, parabens and phenols found in personal care products with pubertal timing in girls and boys. Hum Reprod 34:109–117. [PubMed: 30517665]

Hauser R, Williams P, Altshul L, Korrick S, Peeples L, Patterson DG Jr., et al. 2005. Predictors of serum dioxin levels among adolescent boys in chapaevsk, russia: A cross-sectional pilot study. Environ Health 4:8. [PubMed: 15918907]

Heudorf U, Mersch-Sundermann V, Angerer J. 2007. Phthalates: Toxicology and exposure. Int J Hyg Environ Health 210:623–634. [PubMed: 17889607]

Howdeshell KL, Hotchkiss AK, Gray LE Jr. 2017. Cumulative effects of antiandrogenic chemical mixtures and their relevance to human health risk assessment. Int J Hyg Environ Health 220:179–188. [PubMed: 27923611]

Hoyer BB, Lenters V, Giwercman A, Jonsson BAG, Toft G, Hougaard KS, et al. 2018. Impact of di-2-ethylhexyl phthalate metabolites on male reproductive function: A systematic review of human evidence. Curr Environ Health Rep 5:20–33. [PubMed: 29468520]

Kasper-Sonnenberg M, Koch HM, Wittsiepe J, Bruning T, Wilhelm M. 2014. Phthalate metabolites and bisphenol a in urines from german school-aged children: Results of the duisburg birth cohort and bochum cohort studies. Int J Hyg Environ Health 217:830–838. [PubMed: 24986699]

Kasper-Sonnenberg M, Wittsiepe J, Wald K, Koch HM, Wilhelm M. 2017. Pre-pubertal exposure with phthalates and bisphenol a and pubertal development. PLoS One 12:e0187922.

Kay VR, Bloom MS, Foster WG. 2014. Reproductive and developmental effects of phthalate diesters in males. Crit Rev Toxicol 44:467–498. [PubMed: 24903855]

Koch HM, Calafat AM. 2009. Human body burdens of chemicals used in plastic manufacture. Philos Trans R Soc Lond B Biol Sci 364:2063–2078. [PubMed: 19528056]

Koch HM, Lorber M, Christensen KL, Palmke C, Koslitz S, Bruning T. 2013. Identifying sources of phthalate exposure with human biomonitoring: Results of a 48h fasting study with urine collection and personal activity patterns. Int J Hyg Environ Health 216:672–681. [PubMed: 23333758]

Koch HM, Ruther M, Schutze A, Conrad A, Palmke C, Apel P, et al. . 2017. Phthalate metabolites in 24-h urine samples of the german environmental specimen bank (esb) from 1988 to 2015 and a comparison with us nhanes data from 1999 to 2012. Int J Hyg Environ Health 220:130–141. [PubMed: 27863804]

Lam T, Williams PL, Lee MM, Korrick SA, Birnbaum LS, Burns JS, et al. 2014. Prepubertal organochlorine pesticide concentrations and age of pubertal onset among russian boys. Environ Int 73:135–142. [PubMed: 25118086]

Lebedev AT, Mazur DM, Polyakova OV, Kosyakov DS, Kozhevnikov AY, Latkin TB, et al. 2018. Semi volatile organic compounds in the snow of russian arctic islands: Archipelago novaya zemlya. Environ Pollut 239:416–427. [PubMed: 29679939]

Lee MM, Sergeyev O, Williams P, Korrick S, Zeilert V, Revich B, et al. 2003. Physical growth and sexual maturation of boys in chapaevsk, russia. J Pediatr Endocrinol Metab 16:169–178. [PubMed: 12713253]

Lindsey JC, Ryan LM. 1998. Tutorial in biostatistics methods for interval-censored data. Stat Med 17:219–238. [PubMed: 9483730]

Marsee K, Woodruff TJ, Axelrad DA, Calafat AM, Swan SH. 2006. Estimated daily phthalate exposures in a population of mothers of male infants exhibiting reduced anogenital distance. Environ Health Perspect 114:805–809. [PubMed: 16759976]

Martinchik AN, Baturin AK, Baeva VS, Feoktistova AI, Piatnitskaia IN, Azizbekian GA, et al. 1998. [development of a method of studying actual nutrition according to analysis of the frequency of consumption of food products: Creation of a questionnaire and general evaluation of the reliability of the method]. Vopr Pitan:8–13.

Mazur DM, Detenchuk EA, Sosnova AA, Artaev VB, Lebedev AT. 2021. Gc-hrms with complementary ionization techniques for target and non-target screening for chemical exposure:

Expanding the insights of the air pollution markers in moscow snow. Sci Total Environ 761:144506.

Mieritz MG, Frederiksen H, Sorensen K, Aksglaede L, Mouritsen A, Hagen CP, et al. 2012. Urinary phthalate excretion in 555 healthy danish boys with and without pubertal gynaecomastia. Int J Androl 35:227–235. [PubMed: 22612475]

Mouritsen A, Frederiksen H, Sorensen K, Aksglaede L, Hagen C, Skakkebaek NE, et al. 2013. Urinary phthalates from 168 girls and boys measured twice a year during a 5-year period: Associations with adrenal androgen levels and puberty. J Clin Endocrinol Metab 98:3755–3764. [PubMed: 23824423]

Polyakova OV, Artaev VB, Lebedev capital A C. 2018. Priority and emerging pollutants in the moscow rain. Sci Total Environ 645:1126–1134. [PubMed: 30248837]

Radke EG, Braun JM, Meeker JD, Cooper GS. 2018. Phthalate exposure and male reproductive outcomes: A systematic review of the human epidemiological evidence. Environ Int 121:764–793. [PubMed: 30336412]

Rey RA. 2021. The role of androgen signaling in male sexual development at puberty. Endocrinology 162.

Rockett HR, Colditz GA. 1997. Assessing diets of children and adolescents. Am J Clin Nutr 65:1116S–1122S. [PubMed: 9094907]

Sergeyev O, Burns JS, Williams PL, Korrick SA, Lee MM, Revich B, et al. 2017. The association of peripubertal serum concentrations of organochlorine chemicals and blood lead with growth and pubertal development in a longitudinal cohort of boys: A review of published results from the russian children's study. Rev Environ Health 32:83–92. [PubMed: 28231067]

Shi H, Cao Y, Shen Q, Zhao Y, Zhang Z, Zhang Y. 2015. Association between urinary phthalates and pubertal timing in chinese adolescents. J Epidemiol 25:574–582. [PubMed: 26212725]

Shoaff J, Papandonatos GD, Calafat AM, Ye X, Chen A, Lanphear BP, et al. 2017. Early-life phthalate exposure and adiposity at 8 years of age. Environ Health Perspect 125:097008. [PubMed: 28935615]

Tanner JM, Whitehouse RH. 1976. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Arch Dis Child 51:170–179. [PubMed: 952550]

Turcu AF, Rege J, Auchus RJ, Rainey WE. 2020. 11-oxygenated androgens in health and disease. Nat Rev Endocrinol 16:284–296. [PubMed: 32203405]

Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR Jr., Lee DH, et al. . 2012. Hormones and endocrine-disrupting chemicals: Low-dose effects and nonmonotonic dose responses. Endocr Rev 33:378–455. [PubMed: 22419778]

Williams PL, Sergeyev O, Lee MM, Korrick SA, Burns JS, Humblet O, et al. 2010. Blood lead levels and delayed onset of puberty in a longitudinal study of russian boys. Pediatrics 125:e1088–1096. [PubMed: 20368318]

Witchel SF, Pinto B, Burghard AC, Oberfield SE. 2020. Update on adrenarche. Curr Opin Pediatr 32:574–581. [PubMed: 32692055]

Wormuth M, Scheringer M, Vollenweider M, Hungerbuhler K. 2006. What are the sources of exposure to eight frequently used phthalic acid esters in europeans? Risk Anal 26:803–824. [PubMed: 16834635]

Zhang Y, Cao Y, Shi H, Jiang X, Zhao Y, Fang X, et al. 2015. Could exposure to phthalates speed up or delay pubertal onset and development? A 1.5-year follow-up of a school-based population. Environ Int 83:41–49. [PubMed: 26073845]

Zota AR, Calafat AM, Woodruff TJ. 2014. Temporal trends in phthalate exposures: Findings from the national health and nutrition examination survey, 2001–2010. Environ Health Perspect 122:235–241. [PubMed: 24425099]

Table 1.

Phthalate metabolites and their parent compounds measured in the Russian Children's Study

Parent phthalate	Primary metabolite	Secondary metabolite[a]	LOD[b]	Metabolite name
Low molecular weight (LMW) phthalates				
Diethyl phthalate (dep)	MEP		0.125	Mono-ethyl phthalate
Di-*n*-butyl phthalate (DnBP)	MnBP		0.125	Mono-*n*-butyl phthalate
Di-iso-butylphthalate (DiBP)	MiBP		0.125	Mono-isobutyl phthalate
Butylbenzyl phthalate (BBzP)	MBzP		0.125	Mono-benzyl phthalate
High molecular weight (HMW) phthalates				
Di(2-ethylhexyl) phthalate (DEHP)	MEHP		0.125	Mono(2-ethylhexyl) phthalate
		MEHHP	0.125	Mono(2-ethyl-5-hydroxy-hexyl) phthalate
		MEOHP	0.05	Mono(2-ethyl-5-oxo-hexyl) phthalate
		MECPP	0.125	Mono(2-ethyl-5-carboxy-pentyl) phthalate
Di-isononyl phthalate (DiNP)		MHiNP	0.025	Mono-hydroxy-iso-nonyl phthalate
		MOiNP	0.025	Mono-oxo-iso-nonyl phthalate
		MCOP	0.025	Mono-carboxy-iso-octyl phthalate
Di-iso-decyl phthalate (DiDP)		MHiDP	0.125	Mono-(hydroxy-iso-decyl) phthalate
		MOiDP	0.125	Mono-(oxo-iso-decyl) phthalate
		MCNP	0.125	Mono-(carboxy-iso-nonyl) phthalate
		MCPP[c]	0.05	Mono-(3-carboxypropyl) phthalate

[a]Differing metabolite abbreviations used in European Union are as follows: MEHHP, 5OH-MEHP; MEOHP, 5oxo-MEHP; MECPP, 5cx-MEPP; MECPP; MHiNP, OH-MiNP; MOiNP, oxo-MiNP; MCOP, cx-MiNP; MHiDP, OH-MiDP; MOiDP, oxo-MiDP; MCNP, cx-MiDP.

[b]LOD, limit of detection.

[c]Metabolite of several HMW and LMW phthalates (currently known: DnBP, DnPeP, DnOP, DiNP, DiDP). MCPP has also been abbreviated in previous studies with 3cx-MPP.

Table 2:

Descriptive characteristics of 304 boys with prepubertal urinary phthalate metabolite measurements at entry (age 8–9 years) during 2003–2005 into the Russian Children's Study

Physical Characteristics	Mean (SD)
Height z-score[a]	−0.01±1.02
BMI z-score[a]	−0.36±1.24
Height (cm)	128.6±6.1
BMI (kg/m^2)	15.6±2.0
Prenatal and Birth History[b]	
Mother's age at son's birth	24.1±5.2
Birthweight (kg)	3.3±0.5
Gestational age (wks)	39.0±1.9
Prenatal tobacco smoke exposure (N, %)	148 (50%)
Prenatal alcohol consumption (N, %)	39 (13%)
Breastfed (wks)	26±36
Boys Daily Dietary Intake[b]	
Total calories (calories)	2715±970
Percent calories from carbohydrates	55±7
Percent calories from fat	34±6
Percent calories from protein	12±2
Household Characteristics (N, %)[b]	
Parental education - maximum of either parent	
Technical training/college	274 (91%)
Household income[c]	
≤4,999 Russian rubles/month	99 (33%)
Biological father resides in home	199 (66%)

[a] WHO age-adjusted z-scores: http://www.who.int/childgrowth/en/;

[b] missing information: mother's age at son's birth (n=4), gestational age (n=2), breastfed (n=5), prenatal tobacco smoke (n=6), prenatal alcohol consumption (n=8), dietary information (n=2), parental education (n=3).

[c] Corresponds to ≤172 U.S. dollars/month. Weighted average using average exchange rate in 2003 (1 USD = 30.692 Russian Rubles), 2004 (1 USD=28.814 rubles), 2005 (1 USD=28.284 rubles). Organization for Economic Cooperation and Development (OECD) (2021), Exchange rates (indicator). 2003: doi: 10.1787/037ed317-en (Accessed on 30 August 2021).

Table 3.

Distribution of prepubertal urinary phthalate metabolites among 304 boys in the Russian Children's Study

Urinary Phthalate Metabolite		Percentiles				
Metabolites of Anti-Androgenic Phthalates (AAPs)	**<LOD, N (%)**	**10th**	**25th**	**50th**	**75th**	**90th**
MnBP, ng/mL	0	78.2	125.3	196.5	303.3	447.0
MiBP, ng/mL	0	23.1	34.3	57.1	88.0	148.4
MBzP, ng/mL	1 (<1%)	1.8	2.9	6.2	15.2	30.9
DEHP metabolites						
MEHP, ng/mL	1 (<1%)	4.2	7.1	13.2	23.0	38.9
MEHHP, ng/mL	0	34.5	50.5	79.1	121.7	222.2
MEOHP, ng/mL	0	26.5	41.6	65.8	105.4	179.4
MECPP, ng/mL	0	67.9	104.5	159.4	260.1	442.6
∑DEHP metabolites, μmol/L[a]		0.45	0.71	1.08	1.76	2.91
DiNP metabolites						
MHiNP, ng/mL	0	3.2	4.9	8.2	14.9	27.5
MOiNP, ng/mL	0	1.0	1.7	2.9	5.9	12.4
MCOP, ng/mL	0	1.9	3.3	6.4	12.0	19.5
∑DiNP metabolites, μmol/L[a]		0.02	0.03	0.06	0.10	0.20
∑AAP, μmol/L[a]		1.0	1.6	2.5	3.6	5.4
Metabolites of Other Phthalates						
MEP, ng/mL	0	33.1	51.3	91.2	177.6	375.4
DiDP metabolites						
MHiDP, ng/mL	10 (3%)	0.8	2.0	3.8	8.0	18.2
MOiDP, ng/mL	70 (23%)	<LOD	0.2	0.4	0.8	1.4
MCNP, ng/mL	2 (<1%)	0.3	0.5	0.8	1.4	2.3
∑DiDP metabolites, μmol/L[a]		0.005	0.01	0.02	0.03	0.06
MCPP, ng/mL[b]	0	4.5	6.6	11.0	18.9	27.2

Abbreviations: LOD, limit of detection; MnBP, mono-n-butyl, MiBP, mono-isobutyl; MBzP, monobenzl; DEHP, di(2-ethylhexyl); MEHP, mono(2-ethylhexyl); MEHHP, mono(2-ethyl-5-hydroxy-hexyl); MEOHP, mono(2-ethyl-5-oxo-hexyl); MECPP, mono(2-ethyl-5-carboxy-pentyl); DiNP, di-isononyl; MHiNP, monohydroxy-iso-nonyl; MOiNP, mono-oxo-iso-nonyl; MCOP, mono-carboxy-iso-octyl; MEP, mono-ethyl; diisodecyl (DiDP); MHiDP, mono-(hydroxy-iso-decyl); MOiDP, mono-(oxo-iso-decyl); MCNP, mono-(carboxyiso-nonyl); MCPP, mono-(3-carboxypropyl).

[a]Molar sum of urinary phthalate metabolites.

[b]Metabolite of several HMW and LMW phthalates (currently known: DnBP, DnPeP, DnOP, DiNP, DiDP).

Table 4.

Adjusted mean shifts in age at pubertal onset [months (95% CI)] by quartiles of prepubertal urinary phthalate metabolites in the Russian Children's Study (N=304)

	Testicular Volume > 3 mL[a]		Genitalia Stage ≥ 2[b]		Pubarche Stage ≥ 2[c]	
Urinary phthalate metabolites	**Mean shift (months) 95% CI**	**P-value**	**Mean shift (months) 95% CI**	**P-value**	**Mean shift (months) 95% CI**	**P-value**
Mono-n-butyl (MnBP)[d]						
Quartile 1	Reference		Reference		Reference	
Quartile 2	4.8 (−0.1, 9.7)	0.06	2.9 (−3.2, 9.0)	0.35	4.2 (−2.5, 11.0)	0.22
Quartile 3	−0.5 (−5.7, 4.7)	0.85	2.7 (−3.9, 9.3)	0.42	3.4 (−3.8, 10.6)	0.36
Quartile 4	−0.3 (−5.9, 5.3)	0.92	3.4 (−3.6, 10.4)	0.34	9.3 (1.5, 17.1)	0.02
Trend teste		0.53		0.38		0.03
Mono-isobutyl (MiBP)[f]						
Quartile 1	Reference		Reference		Reference	
Quartile 2	8.5 (3.7, 13.5)	<0.001	6.4 (0.2, 12.6)	0.04	5.9 (−0.9, 12.6)	0.09
Quartile 3	6.4 (1.1, 11.7)	0.02	7.2 (0.5, 13.8)	0.03	10.2 (2.9, 17.5)	0.006
Quartile 4	5.7 (0.2, 11.1)	0.04	6.2 (−0.7, 13.1)	0.08	12.8 (5.3, 20.3)	<0.001
Trend test[e]		0.13		0.11		<0.001
Monobenzyl (MBzP)[g]						
Quartile 1	Reference		Reference		Reference	
Quartile 2	−0.6 (−5.6, 4.3)	0.80	3.2 (−3.0, 9.4)	0.31	4.9 (−1.7, 11.6)	0.15
Quartile 3	5.6 (0.3, 11.0)	0.04	5.7 (−0.9, 12.3)	0.09	15.1 (8.0, 22.2)	<0.001
Quartile 4	5.6 (0.6, 10.7)	0.03**	7.5 (1.1, 13.8)	0.02	14.2 (7.4, 21.0)	<0.001
Trend test[e]		0.006		0.02		<0.001
∑Di(2-ethylhexyl) (DEHP) metabolites[h]						
Quartile 1	Reference		Reference		Reference	
Quartile 2	3.2 (−1.7, 8.1)	0.20	3.7 (−2.5, 9.9)	0.24	8.4 (1.6, 15.3)	0.02
Quartile 3	8.0 (2.9, 13.2)	0.002	8.3 (1.8, 14.7)	0.01	14.1 (7.0, 21.1)	<0.001
Quartile 4	−0.1 (−5.5, 5.2)	0.96	3.6 (−3.1, 10.4)	0.29	10.0 (2.5, 17.4)	0.009
Trend test[e]		0.72		0.19		0.006
∑Di-isononyl (DiNP) metabolites[i]						
Quartile 1	Reference		Reference		Reference	
Quartile 2	4.6 (−0.3, 9.5)	0.07	5.1 (−1.1, 11.2)	0.10	0.3 (−6.4, 7.0)	0.92
Quartile 3	4.5 (−0.7, 9.7)	0.09	6.5 (0.03, 13.0)	0.05	8.9 (1.8, 15.9)	0.01
Quartile 4	5.4 (0.01, 10.7)	0.05*	6.1 (−0.6, 12.7)	0.08	8.4 (1.1, 15.7)	0.02
Trend test[e]		0.07		0.09		0.005
∑Anti-androgenic phthalate metabolites (AAP)[j]						
Quartile 1	Reference		Reference		Reference	
Quartile 2	4.2 (−0.8, 9.2)	0.10	3.4 (−2.8, 9.5)	0.29	6.8 (−0.1, 13.7)	0.05

Urinary phthalate metabolites	Testicular Volume > 3 mL[a] Mean shift (months) 95% CI	P-value	Genitalia Stage ≥ 2[b] Mean shift (months) 95% CI	P-value	Pubarche Stage ≥ 2[c] Mean shift (months) 95% CI	P-value
Quartile 3	4.9 (−0.5, 10.4)	0.08	9.1 (2.4, 15.8)	0.008	9.9 (2.4, 17.3)	0.01
Quartile 4	0.6 (−5.0, 6.2)	0.82	2.4 (−4.5, 9.3)	0.50	10.9 (3.2, 18.7)	0.006
Trend test[e]		0.93		0.37		0.006

Interval-censored survival models adjusted for:

[a] prenatal tobacco smoke exposure, birthweight, breastfed, household income, and urinary specific gravity;

[b] prenatal tobacco smoke exposure, mother's age at son's birth, breastfed, biological father living in home, and urinary specific gravity;

[c] prenatal maternal alcohol intake, and urinary specific gravity.

[d] MnBP quartiles: Q1 12.5 – 125.1; Q2 125.2 – 195.8; Q3 195.9 – 299.8; Q4 299.9 – 1349.3 ng/mL;

[e] Trend tests performed by modeling phthalate metabolite quartiles as an ordinal variable.

[f] MiBP quartiles: Q1 3.3 – 34.2; Q2 34.3 – 56.9; Q3 57.0 – 87.3; Q4 87.4 – 1754.6 ng/mL;

[g] MBzP quartiles: Q1 0.15 – 2.82; Q2 2.83 – 6.12; Q3 6.13 – 15.11; Q4 15.12 – 600.85 ng/mL;

[h] ∑DEHP (mono(2-ethylhexyl, mono(2-ethyl-5-hydroxy-hexyl, mono(2-ethyl-5-oxo-hexyl, and mono(2-ethyl-5-carboxypentyl) phthalates quartiles: Q1 0.13 – 0.71; Q2 0.72 – 1.07; Q3 1.08 – 1.75; Q4 1.76 – 65.55 μmol/L;

[i] ∑DiNP (mono-hydroxy-iso-nonyl, mono-oxo-iso-nonyl, and mono-carboxy-iso-octyl phthalates) quartiles: Q1 0.006 – 0.032; Q2 0.033 – 0.056; Q3 0.057 – 0.102; Q4 0.103 – 1.756 μmol/L;

[j] ∑AAP (MBzP, MiBP, MnBP, [∑DiNP*0.43], and ∑DEHP) quartiles: Q1 0.37 – 1.57; Q2 1.58 – 2.49; Q3 2.50 – 3.58; Q4 3.59 – 76.36 μmol/L.