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Endocrine disrupting chemical exposure and maladaptive behavior during adolescence

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Abstract

Background: Studies suggest that exposure to endocrine disrupting chemicals (EDCs), including phthalates, phenols, and parabens may influence childhood behavior, but the relationship during adolescence has not been assessed.

Objective: We investigated the association between urinary biomarker concentrations of potential EDCs, including some phthalate and bisphenol A replacement chemicals, and behavior in adolescents.

Methods: Participants were from the New Bedford Cohort (NBC), a prospective birth cohort of residents near the New Bedford Harbor Superfund site in Massachusetts. We measured urinary concentrations of 16 phthalate metabolites or replacements, 8 phenols, and 4 parabens in 205 NBC adolescents and estimated associations between select EDCs and adolescent behavior assessed with the Behavior Assessment System for Children, Second Edition -Teacher Rating Scale

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envres.2018.12.053

(BASC-2). Of note, up to 32 of the 205 in our assessment had missing outcome information imputed.

Results: Increased urinary concentrations of the sum of 11 antiandrogenic phthalate metabolites were associated with an increase in maladaptive behaviors (Externalizing Behavior, Behavioral Symptoms Index, and Developmental Social Disorders or DSD), and a decrease in Adaptive Skills. For example, a doubling of urinary concentrations of antiandrogenic phthalate metabolites was associated with an increased risk of Externalizing Behavior (RR=1.04; 95% CI: 1.01–1.08). While associations were generally stronger in males, sex differences were not statistically significant. Urine concentrations of phenols and parabens were not associated with adverse behavior.

Conclusion: Our findings support the importance of exposure to antiandrogenic phthalates during adolescence as a potential correlate of maladaptive behaviors including Externalizing Behavior, DSD behaviors, and decrements in Adaptive Skills.

Keywords

Endocrine disrupting chemicals; Phthalates; Phenols; Parabens; Adolescence; Behavior

1. Introduction

Adolescence is a critical developmental period during which the brain undergoes substantial functional and structural changes. For example, increases in white matter, decreases in gray matter, synaptic pruning, and maturation of the limbic system are characteristic of adolescence and are important to the development of executive function skills characteristic of this period (Casey et al., 2008). Adolescence is also a period of increased risk-taking behavior and emotional reactivity (Steinberg, 2004) and a critical time for the onset of depression and anxiety disorders (Merikangas et al., 2009). Indeed, deficiencies in development during this period may play a role in behavioral and mental health disorders. Changes in the brain and behavioral characteristics observed during adolescence, in part, reflect alterations in sex hormones during puberty (Casey et al., 2008). As such, exposure to factors that interfere with endocrine function during this time period may be particularly detrimental.

Endocrine disrupting chemicals (EDCs) such as phthalates, phenols, and parabens are widely used in a variety of products including food packaging and processing materials, medical products, consumer goods, and personal care products such as cosmetics, soaps and fragrances, resulting in ubiquitous exposure (Braun et al., 2013, 2009; Harley et al., 2016). Neuroendocrine disruption is hypothesized to be one possible mechanism for neurotoxicity of EDCs as they can not only mimic or inhibit hormones but also alter target tissue response to hormonal signaling.

Despite evidence that adolescence is a critical time window for neurodevelopment and likely a vulnerable period for EDC exposure, there is limited research exploring the impact of EDC exposure during this time. However, a substantial number of epidemiologic studies have examined associations of prenatal exposure to phthalates and BPA with behavior. While

results have been mixed, with studies reporting null, adverse, and beneficial associations, overall, the literature suggests adverse impacts on behavior (Ejaredar et al., 2015; Miodovnik et al., 2014; Mustieles et al., 2015). Prospective epidemiologic studies have variously reported increased anxiety, depression, autistic-like behaviors, internalizing and externalizing behaviors, aggressiveness, delinquent behaviors, attention deficit/hyperactivity disorder (ADHD)-like behaviors and attention problems, in children born to mothers with higher urinary concentrations of BPA or select phthalate metabolites during pregnancy (Braun et al., 2009, 2017; Ejaredar et al., 2015; Miodovnik et al., 2014, 2011; Engel et al., 2010; Whyatt et al., 2012; Lien et al., 2015; Kim et al., 2017; Philippat et al., 2017; Perera et al., 2016, 2012; Roen et al., 2015). To date, only one published epidemiologic study has assessed paraben exposure and behavior; in this case, higher maternal urine paraben concentrations in pregnancy were associated with better social behavior at age three years (Philippat et al., 2017).

Several cross sectional studies that have combined a range of ages, including those consistent with adolescence (maximum ages ranging from 11 to 17 years), support the potential for increased adolescent EDC exposure to be associated with adverse behavior. Increased urinary phthalate metabolites in childhood have been associated with autism spectrum disorders (ASDs), Attention Deficit Disorder, ADHD-like behaviors, and learning disabilities (Chopra et al., 2014; Kim et al., 2009; Won et al., 2016; Kardas et al., 2016; Rahbar et al., 2017), while higher childhood urinary BPA concentrations have been associated with ASDs, ADHD, hyperactivity, and poor social behavior (Kardas et al., 2016; Rahbar et al., 2017).

Experimental studies in rodents corroborate these findings. For example, increased adolescent exposures to phthalates and BPA have been associated with changes in brain regions related to regulation of emotion and behavior including the amygdala (with phthalate exposure) (Betz et al., 2013) and the hippocampus and prefrontal cortex (with BPA exposure) (Luo et al., 2013; Wise et al., 2016). Further, in both rats and mice, adolescent phthalate exposure has been associated with anxiety and alterations in play behavior and social interaction (Betz et al., 2013; Carbone et al., 2013; Wang et al., 2016) while adolescent BPA exposure has also been associated with anxiety (Luo et al., 2013; Bowman et al., 2015; Yu et al., 2011; Diaz Weinstein et al., 2013).

In recent years, phthalates used in some applications [e.g. di-2-ethylhexyl phthalate (DEHP) in children's toys] have been replaced by 1,2 cyclohexane dicarboxylic acid, diisononyl ester (DINCH) (Silva et al., 2013). Little is known about the potential human health effects of DINCH exposure but recent studies have reported that a metabolite of DINCH, cyclohexane-1,2-dicarboxylic acid monoisononyl ester (MHINCH), may interfere with the endocrine system in animal models (Campioli et al., 2015), and may alter female reproductive health in ways that are similar to DEHP (Minguez-Alarcon et al., 2016).

Similarly, as the use of BPA in consumer products has decreased in recent years because of concerns about potential human health risks, bisphenol F (BPF) and bisphenol S (BPS) have become commonly used replacements. While there is little published on the human health effects of BPF and BPS, they are structurally similar to BPA and findings from in vitro

studies suggest that their actions and potencies are similar to BPA (Rochester and Bolden, 2015). Further, one study has reported associations between higher prenatal exposure to BPF and increased anxiety and depressive behaviors in rats (Ohtani et al., 2017).

There is a need for more human data to determine whether there are health impacts of these newer replacement chemicals as well as other common EDCs, such as parabens. There is also a need for human studies examining EDC exposure during adolescence, a relatively unstudied period despite likely heightened vulnerability. Given the limited research data available for this critical area, the objective of this study was to examine the relationship between adolescent exposure to commonly occurring EDCs – specifically phthalates, phenols and parabens as well as phthalate and BPA replacement chemicals – and behavioral problems in adolescence and to consider potential sexual dimorphism in associations.

2. Methods

2.1. Study population

We analyzed data collected from an ongoing prospective birth cohort, the New Bedford Cohort (NBC), in which mother-infant pairs were recruited after delivery at St. Luke's Hospital (New Bedford, Massachusetts) from 1993 to 1998 (Korrick et al., 2000). The original aim of the study was to examine the relationship of prenatal organo-chlorine and metal exposures with subsequent neurodevelopment among children living near the New Bedford Harbor Superfund site. Women who were at least 18 years old and vaginally delivered infants available for neonatal examination were eligible to participate. Study children have undergone periodic developmental assessments including extensive neurodevelopmental testing in adolescence.

Between 2008 and 2014, in-person neurodevelopmental testing was done on NBC participants at approximately 15 years of age. Of the 788 newborns enrolled in the NBC, 660 (84%) met eligibility criteria for these assessments and of those, 528 (80%) completed assessments. Eligibility criteria included: residence in the study region (necessary for in-person testing), intact cognition (e.g., study participants with a history of central nervous system cancer or post-traumatic cognitive impairment that precluded standardized testing were excluded), available biomarkers of early chemical exposure, and trackable contact information.

Mid-way through the 15-year follow-up we expanded the research to include exposure assessment for phthalates and phenols. Specifically, 252 participants were asked to provide a spot urine sample at the study office during the neurodevelopmental assessment and, again, approximately one week later (mean: 7 days, range: 1–35 days), during a study home visit.

The study research protocol was reviewed and approved by the human subjects committee of the Brigham and Women's Hospital (Boston, Massachusetts). Written informed consent was obtained from all participating families (parental consent and child assent) before study evaluation. The analysis of de-identified specimens by the Centers for Disease Control and Prevention (CDC) laboratory was determined not to constitute engagement in human subjects research.

2.2. Urine collection

Of 252 NBC adolescents asked to provide two urine samples, 205 (81%) provided at least one urine sample. Of these, 144 (70%) provided two urine samples and the rest collected only one sample either at the study office (n=52) or at a study home visit (n=9). Random spot urines (and field blanks) were collected in sterile, polypropylene urine cups and stored at -20°C until processed in Boston where samples were thawed to room temperature, mixed on a vortex mixer, aliquoted into polypropylene cryovials, and had specific gravity measured before being frozen at -80°C . Specific gravity was measured using a handheld digital refractometer (Pocket PAL-10S, ATACO USA, Inc.).

2.3. Urine phthalate, phenol and paraben measurements

Because of resource limitations, it was not possible to analyze all of the collected urine specimens. Of the 144 adolescents who provided both clinic and home samples, 60 had each urine analyzed separately and the mean concentration was used in our analyses to characterize usual adolescent exposure. For the remaining 84, the concentration from a single pooled sample, combining the study office and home samples, was used in our analyses to characterize usual exposure.

Frozen individual urines, urine pools, field blanks and urine qa/qc samples (for details see Supplemental Methods: Exposure Assessment) were shipped to the CDC (Atlanta, GA) for analysis in two batches (2012 and 2016). The CDC laboratory staff quantified total (free plus conjugated) urine concentrations of 28 possible biomarkers by online solid phase extraction coupled with high performance liquid chromatography-isotope dilution-tandem mass spectrometry (Calafat et al., 2008; Kato et al., 2005; Silva et al., 2007; Ye et al., 2005). These included 11 phthalate metabolites and 8 phenols in batch one. Five additional biomarkers of phthalates or phthalate substitutes, and 4 additional phenols were measured in batch two samples. We focused on a subset of these 28 biomarkers that were of *a priori* interest because of: mechanism of action (phthalates with anti-androgenic activity), shared exposure pathways (phthalates and parabens in personal care products), or limited prior human health research (Braun et al., 2014a; Hannas et al., 2011; Howdeshell et al., 2015; Larsson et al., 2014). The latter category included parabens and replacements for common phthalates such as DEHP (i.e., DINCH) or BPA replacements (i.e., BPS and BPF). As a result, our data analyses focused on 12 phthalate metabolites: mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono(2-ethyl-5-carboxypentyl) phthalate (MECPP), mono-n-butyl phthalate (MBP), mono-iso-butyl phthalate (MiBP), monobenzyl phthalate (MBzP), monoethyl phthalate (MEP), monocarboxy-isooctyl phthalate (MCOP) mono-hydroxyisobutyl phthalate (MHIBP), mono-isononyl phthalate (MNP), and mono-hydroxybutyl phthalate (MHBP); two metabolites of the phthalate replacement DINCH [MHINCH and cyclohexane-1,2-dicarboxylic acid, monocarboxy-isooctyl ester (MCOCH)]; and 7 phenols: BPA, BPF, BPS, butyl paraben (B paraben), ethyl paraben (E paraben), methyl paraben (M paraben), and propyl paraben (P paraben) (additional details regarding exposure assessment are available in the Supplemental Methods: Exposure Assessment). Because batch one (2012) analyses did not include all target biomarkers, urinary concentrations for all targeted analytes were available for 178 (87%) participants with the

balance (n=27) missing concentrations of DINCH metabolites, MHIBP, MNP, MHBP, BPF, BPS, and E-paraben. The limits of detection (LOD) for the target analytes ranged from 0.2 to 1.2 $\mu\text{g/L}$. For values less than the LOD, the instrumental reading values provided by CDC were used in data analyses.

The CDC laboratory methods for urine analyses have excellent sensitivity and reproducibility with coefficients of variation (CVs) ranging from 2.7% to 15% (Silva et al., 2007; Ye et al., 2005, 2006). In addition, study-specific urine qa/qc samples demonstrated excellent reproducibility (See Supplemental Methods: QA/QC Assessment of Biomarker Analyses and Supplemental Tables 1-4). Intraclass Correlation Coefficients (ICCs) of urinary biomarker concentrations for participants who provided two urine samples ranged from 0.05 to 0.93 which is consistent with the expectation of substantial variability in exposure to some phthalates and phenols even within a relatively short time interval (Supplemental Table 5) (Pollack et al., 2016; Valvi et al., 2015).

2.4. Exposure assessment

Review of the toxicology literature identified parent diester phthalates with the capacity to decrease fetal testicular testosterone production in rats, a sensitive and specific measure of anti-androgen activity (Hannas et al., 2011; Howdeshell et al., 2015, 2008). Based on this review, we identified 11 phthalate metabolites derived from antiandrogenic parent compounds: MEHHP, MEHP, MEOHP, MECPP, MBP, MiBP, MBzP, MHiBP, MCOP, MNP, and MHBP. The molar sum of these metabolites was used to estimate Σ Antiandrogenic phthalates ($\mu\text{mol/L}$). Compared to the other anti-androgen phthalates, one (DiNP) had a 0.43 relative impact on fetal testosterone production (Hannas et al., 2011). Thus the two metabolites of DiNP, MCOP and MNP, were down weighted by multiplying their molar concentrations by 0.43 prior to summing. We also created a summary measure for phthalates often found in personal care products (Σ Personal care product, $\mu\text{mol/L}$) as the molar sum of MBP and MEP and a summary measure for total paraben exposure (Σ Parabens, $\mu\text{mol/L}$) as the molar sum of butyl, ethyl, methyl, and propyl paraben. We created a variable for the sum of DEHP metabolites (Σ DEHP, $\mu\text{mol/L}$) as the molar sum of MECPP, MEHHP, MEOHP, MEHP. DINCH, a recent replacement for DEHP in some consumer products, has two primary metabolites, MCOCH and MHINCH; however, because 77% of our measured values for MCOCH were below the LOD and 45% of samples had a value of 0, we did not include MCOCH in analyses. Thus, we focused on eight exposure biomarkers including Σ Antiandrogenic phthalates, Σ Personal care product phthalates, Σ Parabens, Σ DEHP and its replacement's metabolite, MHINCH; because of an *a priori* interest in the potential association of recent replacement chemicals with behavior, we also examined BPA and its replacements, BPS and BPF.

2.5. Behavior assessment

Participants' teachers completed the Behavior Assessment System for Children, Second Edition -Teacher Rating Scale (BASC-2 TRS) a median of approximately 2.5 months (IQR: 4.5 months) after urine was collected. The BASC-2 TRS for adolescents (ages 12–21 years) is a standardized questionnaire designed to assess behavioral and emotional function. It contains 139 questions with the relative frequency of adaptive and problem behaviors

observed during school ranked using a four-point Likert scale; the results are used to generate composite behavior measures. For this analysis, we selected a subset of these behavior measures that have been associated with phthalates and/or phenols in at least some previous studies (Ejaredar et al., 2015; Miodovnik et al., 2014; Mustieles et al., 2015; Inadera, 2015). These included: Externalizing Behaviors (a composite of subscales for hyperactivity, aggression and conduct problems), and Internalizing Behaviors (a composite of subscales for anxiety, depression and somatization). In addition, we assessed the Behavioral Symptoms Index (BSI), a summary of overall level of problem behaviors that integrates subscale measures of hyperactivity, aggression, depression, atypicality, withdrawal, and attention problems. For these measures, a higher score indicates more problem behaviors. Lastly, we assessed adaptive behavior and behaviors related to Autism Spectrum Disorders (ASDs). The BASC-2 TRS measure of Adaptive Skills is a composite of teacher observed subscales for adaptability, social skills, functional communication, leadership, study skills, and activities of daily living. Unlike the maladaptive behavior scales, a lower score on the Adaptive Skills composite scale is adverse. The BASC-2 TRS also provides a measure of Developmental Social Disorders (DSD) which integrates teacher observed maladaptive behaviors associated with ASDs.

The BASC-2 TRS provides raw scores as well as age and sex adjusted T-scores standardized to a mean of 50 with a standard deviation of 10. Due to the potential loss of information that may occur when raw scores are truncated to create T-scores, we used raw scores for our main analyses and adjusted for age and sex in our models.

2.6. Covariates

Parental demographics (race/ethnicity, education, and income), health history, and information about smoking, alcohol intake, and diet during pregnancy were collected in a comprehensive study questionnaire administered to study mothers approximately two weeks postpartum. At the 15-year assessment, pediatric medical records were reviewed and questionnaires were administered to the adolescents and to each adolescent's mother (or primary care giver) to update demographics, lifestyle, and child medical history. A diagnosis of a behavioral disorder was based on having a diagnosis in the pediatric medical record or a parental reported ever diagnosis on the study questionnaire (for example, commonly reported diagnoses included ADHD, anxiety, or a problem requiring behavioral therapy or counseling). Information about medication use for a behavioral disorder was based on parental report, and information about adolescent tobacco, alcohol, and marijuana use was based on self-report using a modified version of the CDC Youth Risk Behavior Questionnaire (Centers for Disease Control and Prevention). Biomarkers of prenatal exposure to chemical contaminants that are available on the NBC were considered as potential covariates. These included cord serum levels of polychlorinated biphenyls (PCBs) and dichlorodiphenyldichloroethylene (DDE); cord blood levels of lead and manganese; and maternal peripartum hair mercury and toenail arsenic levels, measured as described elsewhere (Korrick et al., 2000; Sagiv et al., 2012). In addition, infant and childhood blood lead levels were abstracted from pediatric medical record reports of annual childhood lead exposure screening; the maximum value between ages 12–36 months was calculated. Correlations between biomarkers of EDC exposure in adolescence and biomarkers of

BASC-2 scores were missing for up to 32 participants, some biomarker measures were missing for 27 participants, and some covariate information was missing for 19 participants. We imputed these missing values (PROC MI/MIANALYZE, Unix SAS, version 9.1.4; SAS Institute., Cary, North Carolina) based on 20 imputations using models with all covariates, behavioral outcomes and biomarker concentrations considered in this analysis (SAS/STATA, 2013).

We performed a number of sensitivity analyses. We assessed the influence of imputation by conducting a complete case analysis (n:129–153, depending the number of participants who had exposure measures for each analyte). We analyzed BASC-2 T-scores instead of raw scores for outcomes. We included 12–36-month maximum blood lead levels (median 5.0 and range 1–18 µg/dL, consistent with high childhood lead poisoning rates in the New Bedford area), in models for which ΣAntiandrogenic phthalates or ΣPersonal care product phthalates was the primary exposure. We adjusted for whether adolescents had smoked cigarettes in the past 30 days or ever tried alcohol or marijuana (n=60), and also conducted analyses adjusting for current tobacco smoking (cigarettes, cigars, or pipes) by the adolescent's mother or father (n=97). We also conducted analyses adjusting for prior diagnosis of behavioral problems (n=83), removing those who were on prescription medication for behavioral problems (n=22), removing participants who were the result of a twin pregnancy (n=3), removing influential points (n=2), and removing participants with a BASC-2 F index of 4 or greater (n=6). The F index is a validity indicator that identifies a tendency for excessively negative responses. Influential points were defined as observations that had extreme Cook's Distance or leverage values for each exposure-outcome pair.

3. Results

3.1. Study population

At the time of birth, most study mothers had at least a high school education (n=169, 82%) and were under the age of 30 (n=148, 72%). A substantial minority of study adolescents were non-white (n=81, 40%), were born into low income households (n=66, 32%), had unmarried mothers (n=87, 42%), and mothers who smoked during pregnancy (n=56, 27%) (Table 1). Adolescents included in this analysis were younger and a higher percentage were non-white compared to the 528 participating in the full 15-year follow up, but, otherwise, the two groups did not differ (Supplemental Table 6).

3.2. EDC exposure

The median urine concentrations of ΣAntiandrogenic phthalates, ΣPersonal care product phthalates, ΣDEHP metabolites, and ΣParabens were 0.45, 0.34, 0.13, and 0.35 µmol/L respectively. Median urine concentrations of MHINCH, BPA, BPS, and BPF were 0.30, 1.70, 0.40, and 0.20 µg/L respectively (Table 2). Individual biomarker concentrations among our study participants are similar to those observed in U.S. adolescents in the 2011–2012 National Health and Nutrition Examination Survey (Prevention, 2009).

3.3. Behavior

Respective mean (SD) raw and t-scores for each outcome were as follows: for Externalizing Behavior 147 (33) and 49 (12); for Internalizing Behavior 141 (21) and 47 (8); for BSI 298 (53) and 50 (11); for DSD 13 (7) and 51 (11); and for Adaptive Skills 244 (47) and 49 (10). Of note, for Adaptive Skills (where a lower score is adverse) and DSD measures (where a higher score is adverse), study adolescents' average behavior was slightly worse than the standardization sample (mean T-score of 50) whereas, on average, study participants performed slightly better than standard on Externalizing and Internalizing measures.

In covariate-adjusted models, the Σ Antiandrogenic phthalates was consistently associated with adverse behavior on all outcome measures (Figs. 1 and 2, Table 3). These associations were generally stronger in males than females, but the sex specific differences were not statistically significant (product interaction p-values > 0.05).

3.4. Externalizing behavior

In covariate-adjusted models, we observed a two-fold increase in urinary Σ Antiandrogenic phthalate concentration, was associated with increased Externalizing Behavior (Rate Ratio [RR]: 1.04, 95% CI: 1.01, 1.08). Σ Personal care product phthalate and Σ DEHP were also associated with an increase in Externalizing Behavior (RR: 1.02, 95% CI: 1.00, 1.04 and RR: 1.03, 95% CI: 1.00, 1.06 respectively). Higher urine MINCH concentrations were unexpectedly associated with less Externalizing Behavior in boys but the association was very modest in magnitude (RR: 0.99, 95% CI: 0.98, 1.00) and the sex difference in association was not statistically significant. Urinary concentrations of Σ Parabens, BPA, BPS, and BPF were not associated with externalizing behaviors (Figs. 1 and 2).

3.5. Internalizing behavior

We observed a weak, but significant, adverse association between urinary concentration of Σ Antiandrogenic phthalates and Internalizing Behavior. A two-fold increase of Σ Antiandrogenic phthalate urinary concentration was associated with a 1.03 (95% CI: 1.00, 1.05) increase in the rate of internalizing behaviors. Urinary concentrations of Σ Personal care product phthalates, Σ DEHP, Σ Parabens, MHINCH, BPA, BPS, and BPF were not associated with internalizing behaviors in males or females (Figs. 1 and 2).

3.6. Behavioral Symptoms Index (BSI)

Each two-fold increase in urinary Σ Antiandrogenic phthalate concentration was associated with an increase in overall problem behaviors measured with the BSI (RR: 1.03, 95% CI: 1.01, 1.06). Σ DEHP was also associated with an increase in the BSI (RR: 1.02, 95% CI: 1.00, 1.05). Similar to Externalizing Behaviors, higher urine MINCH concentrations were modestly associated with better BSI scores in boys (RR: 0.99, 95% CI: 0.99, 1.00) but the sex difference in association was not statistically significant. Urinary concentrations of Σ Personal care product phthalates, Σ Parabens, BPA, BPS, and BPF were not associated with the BSI (Figs. 1 and 2).

3.7. Adaptive skills

In contrast to the other behavioral measures, a *lower* score on the Adaptive Skills measure is adverse. A two-fold increase in urine Σ Antiandrogenic phthalate concentration was associated with poorer raw scores for Adaptive Skills (β : -9.9, 95% CI: -16.7, -3.1), in the context of an outcome with a mean of 244 (SD: 47), representing 21% of a standard deviation decrease in Adaptive Skills. While sex differences were not statistically significant, associations were stronger in males than females. In males, a two-fold increase in urinary Σ Antiandrogenic phthalate concentration was associated with a 12.5 point decrease in the raw score for Adaptive Skills (95% CI: -21.9, -3.2) while in females the association was smaller (β -7.6, 95% CI: -16.3, 1.1) (Table 3). Σ EDEHP was also associated with a lower Adaptive Skills raw score (β : -7.3, 95% CI: -13.1, -1.4) that was stronger in males (β : -12.0, 95% CI: -20.2, -3.8) than in females (β : -3.4, 95% CI: -10.9, 4.0). We did not observe associations between urinary concentrations of any of the other EDCs and Adaptive Skills.

3.8. Developmental Social Disorders (DSD)

Adverse associations were observed between a two-fold increase in urinary Σ Antiandrogenic phthalate concentration and DSD raw score (β : 1.4, 95% CI: 0.4, 2.5) as well as Σ EDEHP (β : 1.1, 95% CI: 0.1, 2.0) in the context of an outcome with a mean of 13 (SD: 7), representing 20% and 16% of a standard deviation increase in DSD respectively (Table 3). We did not observe DSD associations with any of the other EDCs.

3.9. Sensitivity analyses

Findings were essentially unchanged in complete case analyses (versus our main analyses imputing missing data) (Supplemental Table 7). Further, there were no meaningful changes to our results when we used T-scores for behavioral outcomes instead of raw scores, excluded those on prescription medication for behavioral disorders, adjusted for a diagnosis of a behavioral disorder, adjusted analyses of Σ Antiandrogenic and Σ Personal care product phthalates for maximum 12–36-month blood lead levels, removed participants that were part of a twin pregnancy, removed participants with an elevated BASC-2 F index, or removed influential points (Supplemental Table 7). Similarly, findings were essentially the same after adjustment for adolescent substance use (cigarette smoking, alcohol, or marijuana use) or adjustment for tobacco smoking by a parent (see Supplemental Tables 8 and 9 for representative examples using Σ Antiandrogenic phthalate concentrations).

4. Discussion

To the best of our knowledge, this is the first study to examine associations between select EDCs and behavior focused specifically on exposures and outcomes during adolescence. We found evidence that exposure during adolescence to several of the EDCs evaluated, particularly antiandrogenic phthalates, was associated with increased behavioral problems with the strongest correlates observed for measures of Externalizing Behavior, Adaptive Skills, and DSD. Σ EDEHP was also associated with these outcomes, which is consistent with the fact that DEHP metabolites are a subset of the Σ Antiandrogenic phthalates. While some of these associations were stronger in males, differences between sexes were not statistically

significant in our relatively modest sample size. These findings highlight the potential for select EDCs to be correlates of important behavioral disorders (e.g., the DSD score reflects behavioral deficiencies in Autism Spectrum Disorders) as well as diminished adaptability, an important skill that has been rarely, if ever, studied in the environmental epidemiology literature.

Other published studies typically have not considered mechanistically based exposure groupings. Our analyses found the strongest associations with the sum of antiandrogenic phthalates, supporting the potential value of mechanism of action as the basis for assessing impact of exposure to chemical mixtures.

Again, to the best of our knowledge, there are no published epidemiologic studies assessing the potential behavioral correlates of phthalate replacements (e.g., DINCH) or BPA replacements (e.g., BPS, BPF) and only one that assesses the behavioral correlates of parabens (Philippat et al., 2017). The comprehensive exposure measures available in the NBC allowed us to explore associations with these relatively unstudied potential EDCs. Although we did not observe associations between urinary biomarkers of most of these newer EDCs with behavior, higher urine MINCH concentrations were associated with lower (better) Externalizing Behavior and BSI scores in boys but the associations were very modest in magnitude. This unexpected finding may reflect residual confounding as it is plausible that confounding patterns may vary between historically prevalent EDCs (for which we observed adverse associations) and their more recent replacements. In addition, urine concentrations of these newer chemical biomarkers were generally low in our population decreasing the sensitivity of our analyses (Table 2). Still, this is an important initial exploration of the potential health impacts of phthalate and BPA substitutes. Larger prospective studies are needed to assess human health risks of these substitutes.

The epidemiologic literature on EDC exposure and behavior has predominantly focused on prenatal and early life exposure, and findings vary depending on the outcome measure as well as timing of its assessment. Prenatal exposures to both phthalates and BPA have been associated with aspects of externalizing and internalizing behaviors in some studies (Engel et al., 2010; Lien et al., 2015; Philippat et al., 2017; Perera et al., 2016, 2012; Roen et al., 2015; Harley et al., 2013) but not all (Braun et al., 2009, 2017, 2014b; Evans et al., 2014). Where sex-specific analyses have been done, associations tend to be stronger in boys (Braun et al., 2009, 2017, 2014b; Perera et al., 2016, 2012; Roen et al., 2015; Harley et al., 2013; Evans et al., 2014). Although our sample size may not have been large enough to detect statistically significant effect modification of adolescent exposure impacts by sex, our findings suggest the possibility of increased susceptibility among males that warrants future study.

This analysis was nested in an ongoing prospective cohort study with 205 or 81% of those eligible – by enrolling during the urine collection phase of the study – participating. However, this represented only 39% of all adolescents (n=528) in the full study. For the most part, the characteristics of adolescents (and mothers) included in our analytic sample did not differ substantially from that of the entire sample making selection bias an unlikely explanation of findings (Supplemental Table 6).

One of the fundamental challenges to studying phthalates, phenols and parabens is that their short elimination half-lives, combined with the episodic nature and short-term variability in exposure, mean a single biomarker measurement in urine may not accurately characterize a participant's typical exposure over time and could result in exposure misclassification. However, studies have shown that urinary concentrations of certain phthalate metabolites, particularly MEP, MnBP, MiBP, and MBzP, have fair to good correlation over time (Adibi et al., 2008; Ferguson et al., 2014; Watkins et al., 2014; Teitelbaum et al., 2012). Further, our study collected urine samples from two different time points from most participants, allowing us to better characterize an individual's average or usual adolescent exposure; the importance of this is underscored by the variability of EDC concentrations between urine samples collected, on average, one week apart (Supplemental Table 5).

Lastly, we focused on an *a priori* subset of multiple possible exposure biomarkers (and a subset of *a priori* outcome measures) to limit the risk of type 1 error in our analyses. Furthermore, most of the chemical biomarkers we measured demonstrated excellent laboratory reproducibility; concentrations of the few with higher analytic variability (for example, MEHP and butyl paraben) may reflect larger measurement error (Supplemental Tables 1-4). However, assuming random exposure misclassification, our associations would be biased to the null.

Another important challenge is the potential for reverse causation, especially given the cross sectional design of this analysis. Particular areas of concern include diet and personal care product use, as both are sources of phthalate and BPA exposures, and existing behavioral problems may influence diet or personal care product use habits (Oddy et al., 2009; Trapp et al., 2016). We had detailed diary information regarding these factors at the time of urine collection and were able to adjust for diet and personal care product use in our analysis to reduce the possibility of reverse causation. Of note, these adjustments did not impact associations; for example, the estimate for the association between urinary Σ Antiandrogenic concentrations and externalizing behaviors remained 1.04 (95% CI: 1.01, 1.08) regardless of whether these covariates were included in the model, suggesting that reverse causation by these factors was unlikely to impact our findings. We did not have data available about participants' prenatal phthalate and phenol exposures, however previous studies suggest that there is weak within person correlation between prenatal and childhood exposure to phthalates as well as BPA (Stacy et al., 2016; Shoaff et al., 2017). Analogous or weaker, correlations are likely in our study because it spans an even wider time interval (prenatal to adolescent period). As a consequence, it is unlikely that prenatal exposures would confound observed associations with adolescent exposures.

5. Conclusions

Behavioral problems in adolescents are both prevalent and costly. Approximately 13–20% of adolescents between the ages of 12–18 years experience an emotional, mental, or behavioral disorder in a given year (Merikangas et al., 2010; Perou et al., 2013). In the United States, mental and behavioral disorders in people under the age of 24 result in estimated \$247 billion in annual costs, which includes health care costs, special services (special education, juvenile justice) and loss of productivity (Perera et al., 2016; Perou et al., 2013). Our

findings support the potential importance of exposures during adolescence, especially to antiandrogenic phthalates, as correlates of maladaptive behaviors including Externalizing Behaviors, decrements in Adaptive Skills, and DSD behaviors. This assessment of the hypothesized relation of select EDC exposures with adolescent behavior provides valuable initial insight into potential risk factors for behavioral disorders in this age group.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Rate Ratio (RR)^a for BASC-2^b Internalizing Behavior, Externalizing Behavior, and Behavioral Symptoms Index raw scores associated with a two-fold ($\log_2$) increase in urinary concentrations of biomarkers of phthalates and DINCH in 205^c New Bedford Cohort adolescents. ^aAdjusted for maternal characteristics at birth: age, marital status, education, household income, and alcohol intake and smoking during pregnancy; child: race, sex, age at BASC-2, and, during the 24 h prior to urine collection, personal care product use, fast food consumption, and food or drink microwaved in plastic. ^b Behavior Assessment System for Children, Second Edition -Teacher Rating Scale ^c Sample size of 205 after imputation for missing data. ^dConcentrations were calculated as follows: Σ Antiandrogen phthalates ($\mu\text{mol/L}$): molar sum of MEHHP, MEHP, MEOHP, MECPP, MBP, MiBP, MBzP, MHBP, MCOP, MNP, and MHBP (MCOP and MNP, were down weighted by multiplying their molar concentrations by 0.43 prior to summing to reflect the potency of their parent compound relative to the other anti-androgenic phthalates); Σ Personal Care Product phthalates ($\mu\text{mol/L}$): molar sum of MBP and MEP; Σ DEHP metabolites ($\mu\text{mol/L}$): molar sum of MECPP, MEHHP, MEOHP, MEHP. ^e BSI =Behavioral Symptoms Index.

Fig. 2. Rate Ratio (RR)^a for BASC-2^b Internalizing Behavior, Externalizing Behavior, and Behavioral Symptoms Index raw scores associated with a two-fold (log₂) increase in urinary phenol concentrations in 205^c New Bedford Cohort adolescents. ^aAdjusted for maternal characteristics at birth: age, marital status, education, household income, and alcohol intake and smoking during pregnancy; child: race, sex, age at BASC-2, and, during the 24 h prior to urine collection, personal care product use, fast food consumption, and food or drink microwaved in plastic. ^b Behavior Assessment System for Children, Second Edition - Teacher Rating Scale ^c Sample size of 205 after imputation for missing data. ^dΣParabens (μmol/L) is the molar sum of butyl-, ethyl-, methyl-, and propyl-paraben. ^e BSI =Behavioral Symptoms Index.

Table 1

Distribution of BASC-2^a teacher rating scale t-scores for externalizing and internalizing behavior by characteristics of 205 New Bedford Cohort adolescents with urine EDC measures.

	n (%)	Mean externalizing behavior (SD)	Mean internalizing behavior (SD)
BASC-2 T-score			
	174 (85) ^b	49 (12)	47 (8)
BASC-2 raw score			
	174 (85) ^b	147 (33)	141 (21)
Missing BASC-2	31 (15) ^b	–	–
Maternal characteristics at time of child's birth			
Age (years)			
<20	21 (10)	48 (13)	47 (7)
20–29	127 (62)	50 (12)	47 (10)
30	57 (28)	48 (12)	45 (5)
Missing	0 (0)	NA	NA
Household income (\$/year)			
<20,000	66 (32)	51 (14)	48 (9)
20–<40,000	70 (34)	50 (13)	46 (7)
40–<75,000	53 (26)	47 (9)	46 (9)
75,000	10 (5)	47 (7)	46 (7)
Missing	6 (3)	44 (2) ^c	45 (4) ^c
Marital status			
Married	106 (52)	48 (9)	46 (8)
Unmarried	87 (42)	51 (15)	47 (8)
Missing	12 (6)	47 (7) ^c	49 (10) ^c
Education			
<High School	30 (15)	55 (16) [*]	46 (8)
High School or more	169 (82)	48 (11)	47 (8)
Missing	6 (3)	44 (2) ^c	45 (4) ^c
Smoking during pregnancy			
No	134 (65)	47 (8) [*]	46 (8)

	n (%)	Mean externalizing behavior (SD)	Mean internalizing behavior (SD)
Yes	56 (27)	54 (17)	48 (8)
Missing	15 (7)	52 (15) ^c	48 (13) ^c
Alcohol consumption during pregnancy			
No	141 (69)	48 (10)	46 (8)
Yes	38 (19)	52 (17)	47 (9)
Missing	26 (13)	51 (12) ^c	48 (12) ^c
Child characteristics during adolescence			
Gender			
Male	93 (45)	51 (14) [*]	44 (5) [*]
Female	112 (55)	47 (10)	48 (10)
Missing	0 (0)	NA	NA
Race			
Non-Hispanic White	124 (60)	48 (11)	46 (7)
Non White	81 (40)	51 (14)	48 (10)
Missing	0 (0)	NA	NA
Age at BASC (years)			
14–15	118 (58)	49 (10)	46 (9)
16–17	56 (27)	50 (15)	47 (8)
Missing	31 (15)	^d –	^d –
Personal care product use^e			
<12 products/day	154 (75)	49 (13)	46 (8)
12+products/day	50 (24)	48 (9)	47 (9)
Missing	1 (0.5)	^d –	^d –
Fast food consumption^e			
0 servings/day	98 (48)	49 (11)	46 (8)
1 serving/day	93 (45)	48 (11)	47 (9)
> 1 serving/day	13(6)	55 (22)	46 (5)
Missing	1 (0.5)	^d –	^d –
Food or drink microwaved in plastic^e			

	n (%)	Mean externalizing behavior (SD)	Mean internalizing behavior (SD)
0 servings/day	177 (86)	49 (12)	47 (8)
1+servings/day	27 (13)	51 (11)	47 (9)
Missing	1 (0.5)	— ^d	— ^d
Diagnosis of a behavioral disorder^f			
No	122 (60)	47 (11)	46 (8)
Yes	83 (40)	52 (13)	48 (8)
Missing	0 (0)	NA	NA
Use of medication for a behavioral disorder^g			
No	183 (89)	49 (12) [*]	46 (8)
Yes	22 (11)	53 (11)	48 (12)
Missing	0 (0)	NA	NA
Adolescent substance use^h			
No	145 (71)	47 (8) [*]	46 (8)
Yes	60 (29)	54 (17)	48 (9)
Missing	0 (0)	NA	NA
Parental tobacco smokingⁱ			
No	107 (52)	46 (7) [*]	45 (7) [*]
Yes	97 (47)	52 (14)	48 (9)
Missing	1 (0.5)	42 (NA)	40 (NA)
Peak childhood blood lead (µg/dL)^j			
5	97 (47)	49 (13)	47 (9)
> 5	81 (40)	50 (11)	46 (7)
Missing	27 (13)	46 (8)	46 (7)

^aBehavior Assessment System for Children, Second Edition -Teacher Rating Scale.

^b174 participants had scores for Internalizing Behavior (31 missing), and 173 had scores for Externalizing Behavior (32 missing).

^cOf those missing covariate information, the following had non-missing BASC-2 scores: 5/6 missing household income, 10/12 missing marital status, 5/6 missing maternal education, 22/26 missing alcohol consumption during pregnancy, 12/15 missing smoking during pregnancy.

^dAll of those missing covariate information were missing BASC scores.

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^e Average from two 24-h diary reports (or one 24-h diary report if only one urine sample provided) regarding personal care product use, fast food consumption, and heating food or drink in the microwave using plastic containers.

^f Pediatric medical record or parental reported diagnosis of a behavioral disorder.

^g Parental reported child use of medication for a behavioral disorder.

^h Adolescent report of having smoked a cigarette in the past 30 days or ever having tried marijuana or alcohol.

ⁱ Current tobacco smoking by a parent (cigarette, cigar, or pipe).

^j Peak childhood blood lead levels between the ages of 1–3 years.

* ANOVA p-value < 0.05.

Distribution of urinary concentrations of select biomarkers of phthalates, phenols, and their substitutes among 205 adolescent participants in the New Bedford Cohort who collected urine in 2011–2014.

Table 2

Biomarker ^a	5th Percentile	25th percentile	50th percentile	75th percentile	95th percentile	LOD (µg/L)	Below LOD N (%)
ΣAntidrogen phthalates ^{b,c}	0.11	0.26	0.45	0.71	1.41	–	–
ΣPersonal care product phthalates ^b	0.07	0.18	0.34	0.81	2.36	–	–
ΣDEHP metabolites ^b	0.03	0.08	0.13	0.19	0.50	–	–
ΣParabens ^{b,c}	0.03	0.05	0.35	1.15	5.83	–	–
MHINCH ^c	0.00	0.10	0.30	0.50	1.10	0.4	113 (55)
MCOCH ^c	0.00	0.00	0.10	0.30	0.70	0.5	158 (77)
BPA	0.50	1.00	1.70	2.80	6.70	0.4/0.2 ^d	3 (1)
BPS ^c	0.10	0.20	0.40	0.80	2.30	0.1	2(1)
BPF ^c	0.00	0.10	0.20	0.60	5.50	0.2	71 (35)
Components of summed chemical biomarkers measures							
MIHP	2.1	6.6	11.5	19.3	38.0	0.2/0.8 ^d	1 (0.5)
MBzP	1.3	4.5	9.3	17.8	64.3	0.3	0 (0)
MEHP	0.1	0.7	1.5	3.2	9.7	0.5/0.8 ^d	52 (25)
MEHHP	2.2	6.2	10.5	17.4	45.4	0.2/0.4 ^d	0 (0)
MEOHP	1.6	4.8	7.7	11.7	33.7	0.2	0 (0)
MECPP	5.2	11.6	18.5	28.1	70.8	0.2/0.4 ^d	0 (0)
MCOP	7.4	26.0	49.4	103.0	214.0	0.2/0.3 ^d	0 (0)
MNP ^c	0.2	0.7	1.6	4.3	15.1	0.9	55 (27)
MHBp ^c	0.2	0.7	1.5	2.9	6.6	0.4	17 (8)
MHBp ^c	1.1	2.3	4.0	7.3	17.5	0.4	2(1)
MBP	2.4	8.5	16.0	24.3	53.6	0.4	0 (0)

Biomarker ^a	5th Percentile	25th percentile	50th percentile	75th percentile	95th percentile	LOD (µg/L)	Below LOD N (%)
MEP	7.9	23.9	45.0	122.0	423.0	0.6/1.2 ^d	0 (0)
B-Paraben	0.0	0.0	0.2	0.5	4.1	0.2/0.1 ^d	91 (44)
E-Paraben ^c	0.2	0.4	0.7	2.1	29.0	1.0	103 (50)
M-Paraben	3.6	7.5	42.4	140.2	597.6	1.0	0 (0)
P-Paraben	0.3	1.0	3.7	18.6	116.7	0.2/0.1	0 (0)

^aUnits for sums are µmol/L; units for individual biomarkers are µg/L.

^bConcentrations were calculated as follows: ΣAntiandrogen phthalates (µmol/L): molar sum of MEHHP, MEHP, MEOHP, MECPP, MBP, MiBP, MBzP, MHBP, MCOP, MNP, and MHBP (MCOP and MNP, were down weighted by multiplying their molar concentrations by 0.43 prior to summing to reflect the potency of their parent compound relative to the other anti-androgenic phthalates); ΣPersonal Care Product phthalates (µmol/L): molar sum of MBP and MEP; ΣDEHP metabolites (µmol/L): molar sum of MECPP, MEHHP, MEOHP, MEHP; ΣParabens (µmol/L): molar sum of B-Paraben, E-Paraben, M-Paraben, and P-Paraben.

^cBecause some chemicals were not measured in the first batch of CDC analyses, ΣAntiandrogen phthalates, ΣParabens, MHINCH, MCOCH, BPS, BPF, MNP, MHBP, and E-paraben concentrations were only available for 178 of the 205 participants.

^dLODs differed between the two CDC analysis batches: 2012 LOD/2016 LOD.

Table 3

Linear regression models^a of the difference in raw BASC-2^b scores (Adaptive Skills and Developmental Social Disorders) associated with a two-fold increase in urine concentrations of select biomarkers^c among 205^d New Bedford Cohort adolescents.

	Overall β (95% CI)	Males (n=93) β (95% CI)	Females (n=112) β (95% CI)
Adaptive skills ^e			
Σ Antiandrogen phthalates	-9.88 (-16.68, -3.09) ^f	-12.52 (-21.85, -3.19) ^e	-7.59 (-16.30, 1.11)
Σ Personal care product phthalates	-3.87 (-8.47, 0.73)	-3.58 (-9.71, 2.56)	-4.14 (-10.76, 2.48)
Σ DEHP metabolites	-7.26 (-13.13, -1.39) ^f	-12.00 (-20.19, -3.81) ^f	-3.44 (-10.91, 4.02)
Σ Parabens	-1.44 (-4.53, 1.64)	-1.90 (-6.35, 2.56)	-1.00 (-5.07, 3.06)
MHINCH	0.16 (-1.57, 1.89)	0.90 (-2.01, 3.82)	-0.16 (-2.31, 1.99)
BPA	0.77 (-3.59, 5.13)	-1.80 (-11.54, 7.94)	1.29 (-3.24, 5.81)
BPS	-1.63 (-5.17, 1.92)	3.25 (-5.52, 12.01)	-2.79 (-6.42, 0.84)
BPF	-0.50 (-1.64, 0.64)	-0.82 (-2.80, 1.17)	-0.28 (-1.72, 1.16)
Developmental Social Disorders (DSD) ^e			
Σ Antiandrogen phthalates	1.44 (0.35, 2.54) ^f	1.87 (0.41, 3.33) ^f	1.07 (-0.38, 2.52)
Σ Personal care product phthalates	0.58 (-0.18, 1.35)	0.56 (-0.45, 1.57)	0.60 (-0.46, 1.67)
Σ DEHP metabolites	1.08 (0.13, 2.03) ^f	1.81 (0.50, 3.12) ^f	0.49 (-0.73, 1.71)
Σ Parabens	0.13 (-0.38, 0.65)	0.18 (-0.56, 0.92)	0.09 (-0.58, 0.77)
MHINCH	-0.07 (-0.34, 0.20)	-0.25 (-0.72, 0.22)	0.01 (-0.32, 0.35)
BPA	-0.03 (-0.73, 0.68)	0.22 (-1.37, 1.81)	-0.08 (-0.80, 0.65)
BPS	0.30 (-0.26, 0.86)	-0.54 (-1.93, 0.84)	0.50 (-0.08, 1.08)
BPF	0.05 (-0.14, 0.23)	0.02 (-0.31, 0.35)	0.07 (-0.17, 0.30)

^a Adjusted for maternal characteristics at birth: age, marital status, education, household income, and alcohol intake and smoking during pregnancy; child: race, sex, age at BASC-2, and, during the 24 h prior to urine collection, personal care product use, fast food consumption, and food or drink microwaved in plastic.

^b Behavior Assessment System for Children, Second Edition -Teacher Rating Scale.

^c Concentrations were calculated as follows: Σ Antiandrogen phthalates ($\mu\text{mol/L}$): molar sum of MEHHP, MEHP, MEOHP, MECPP, MBP, MiBP, MBzP, MHBP, MCOP, MNP, and MHPBP (MCOP and MNP, were down weighted by multiplying their molar concentrations by 0.43 prior to summing to reflect the potency of their parent compound relative to the other anti-androgenic phthalates); Σ Personal Care Product phthalates ($\mu\text{mol/L}$): molar sum of MBP and MEP; Σ DEHP metabolites ($\mu\text{mol/L}$): molar sum of MECPP, MEHHP, MEOHP, MEHP; Σ Parabens ($\mu\text{mol/L}$): molar sum of B-Paraben, E-Paraben, M-Paraben, and P-Paraben. Units for sums are $\mu\text{mol/L}$; units for individual biomarkers are $\mu\text{g/L}$.

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^d Sample size of 205 after imputation for missing data.

^e A lower score on Adaptive Skills and a higher score on DSD indicate more maladaptive behavior.

^f p-value< 0.05.