



Bisphenol A exposure and behavioral problems among inner city children at 7–9 years of age



Emily L. Roen^{a,b}, Ya Wang^{b,c}, Antonia M. Calafat^d, Shuang Wang^{b,c}, Amy Margolis^{b,e}, Julie Herbstman^{a,b}, Lori A. Hoepner^{a,b}, Virginia Rauh^{b,f}, Frederica P. Perera^{a,b,*}

^a Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, 722W. 168th St., New York, NY 10032, USA

^b Columbia Center for Children's Environmental Health, Columbia University, 722 W. 168th St., New York, NY 10032, USA

^c Department of Biostatistics, Mailman School of Public Health, Columbia University, 722 W. 168th St., New York, NY 10032, USA

^d National Center for Environmental Health, Centers for Disease Control and Prevention, 4770 Buford Hwy, MS F53, Atlanta, GA 30341, USA

^e Division of Child & Adolescent Psychiatry and the Center for Developmental Neuropsychiatry, Department of Psychiatry, the New York State Psychiatric Institute and the College of Physicians and Surgeons, Columbia University, 1051 Riverside Drive, New York, NY 10032, USA

^f The Heilbrunn Department of Population and Family Health, Columbia University, 60 Haven Avenue, New York, NY 10032, USA

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ABSTRACT

Background: Bisphenol A (BPA) is a ubiquitous endocrine disrupting compound. Several experimental and epidemiological studies suggest that gestational BPA exposure can lead to neurodevelopmental and behavioral problems in early-life, but results have been inconsistent. We previously reported that prenatal BPA exposure may affect child behavior and differently among boys and girls at ages 3–5 years.

Objectives: We investigated the association of prenatal and early childhood BPA exposure with behavioral outcomes in 7–9 year old minority children and hypothesized that we would observe the same sex-specific pattern observed at earlier ages.

Methods: African-American and Dominican women enrolled in an inner-city prospective cohort study and their children were followed from mother's pregnancy through children's age 7–9 years. Women during the third trimester of pregnancy and children at ages 3 and 5 years provided spot urine samples. BPA exposure was categorized by tertiles of BPA urinary concentrations. The Child Behavioral Checklist (CBCL) was administered at ages 7 and 9 to assess multiple child behavior domains. Associations between behavior and prenatal (maternal) BPA concentrations and behavior and postnatal (child) BPA concentration were assessed via Poisson regression in models stratified by sex. These models accounted for potential confounders including prenatal or postnatal urinary BPA concentrations, child age at CBCL assessment, ethnicity, gestational age, maternal intelligence, maternal education and demoralization, quality of child's home environment, prenatal environmental tobacco smoke exposure, and prenatal mono-n-butyl phthalate concentration.

Results: The direction of the associations differed between boys and girls. Among boys ($n=115$), high prenatal BPA concentration (upper tertile vs. lower two tertiles) was associated with increased internalizing ($\beta=0.41$, $p < 0.0001$) and externalizing composite scores ($\beta=0.40$, $p < 0.0001$) and with their corresponding individual syndrome scales. There was a general decrease in scores among girls that was significant for the internalizing composite score ($\beta=-0.17$, $p=0.04$) ($n=135$). After accounting for possible selection bias, the results remained consistent for boys. Conversely, high postnatal BPA concentration was associated with increased behaviors on both the internalizing composite ($\beta=0.30$, $p=0.0002$) and externalizing composite scores ($\beta=0.33$, $p < 0.0001$) and individual subscores in girls but fewer symptoms in boys. These results remained significant in girls after accounting for selection bias.

Conclusion: These results suggest BPA exposure may affect childhood behavioral outcomes in a sex-specific manner and differently depending on timing of exposure.

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1. Introduction

Mental and behavioral disorders in children are a major and growing public health concern because of their increasing prevalence, early onset, and impact on the child, family, and

* Corresponding author at: Columbia Center for Children's Environmental Health, Columbia University, 722W. 168th St., New York, NY 10032, USA.

Fax: +1 212 544 1943.

E-mail address: fpp1@columbia.edu (F.P. Perera).

community (Perou et al., 2013). On average, 17 percent of young people experience an emotional, mental, or behavioral disorder (Merikangas et al., 2010). Attention-deficit/hyperactivity disorder (ADHD) is the most common mental disorder in children followed by behavior/conduct problems, anxiety and depression (Perou et al., 2013). These disorders affect children's ability to learn and their future well-being. In the United States the annual cost of mental and behavioral disorders in persons ≤ 24 years of age is \$247 billion or \$2380 per person (Eisenberg and Neighbors, 2007; Perou et al., 2013). Multiple social and environmental factors are known to play a role in the etiology of these disorders (Canfield et al., 2003; Grandjean et al., 1997; Perera et al., 2013).

Bisphenol A (BPA) is a ubiquitous endocrine disrupting chemical that has been associated, in both animals and humans, with a wide range of health effects, including behavioral problems in children (Braun et al., 2011b; Braun et al., 2009; Harley et al., 2013; Perera et al., 2012). BPA is commonly used to manufacture polymers found in food and drink containers, certain dental sealants (Maserejian et al., 2012), recycling thermal paper (Vandenberg et al., 2007), medical devices and receipts (Biedermann et al., 2010; Geens et al., 2011). According to a national survey, 93 percent of those sampled had detectable levels of BPA in their urine; and higher BPA urinary concentrations were seen among women and low-income individuals (Calafat et al., 2008; Nelson et al., 2012).

Experimental studies have reported associations between BPA exposure and sex-specific changes in brain structure, function and behavior, including loss of sexual dimorphism in animals (Cox et al., 2010; Kundakovic et al., 2013; Nakagami et al., 2009; Palanza et al., 2008; Patisaul et al., 2006; Patisaul et al., 2007; Rubin et al., 2006; Wolstenholme et al., 2011). There is evidence that these effects may be occurring via changes in gene expression and DNA methylation in estrogen signaling pathways and estrogen receptors in a sex-specific, dose-dependent manner (Kundakovic et al., 2013; Naciff et al., 2002; Vandenberg et al., 2009; Wetherill et al., 2007). Epidemiologic studies have reported sex-specific changes in child behavior with increased prenatal BPA exposure (Braun et al., 2011b; Braun et al., 2009; Harley et al., 2013; Perera et al., 2012). However, these associations and sex-specific relationships observed in epidemiological studies have not always been consistent. For example, in our previous analysis of behavioral symptoms on the Child Behavior Checklist (CBCL) at ages 3–5 years in our New York City (NYC) cohort, prenatal BPA urinary concentrations were associated with significant increases in emotional reactivity, and aggression and borderline significant increases in externalizing and internalizing symptoms in boys, and a decrease in anxiety/depression symptoms, aggressive behavior, and internalizing symptoms in girls (Perera et al., 2012). Harley et al. reported significant and positive associations between prenatal BPA urinary concentrations and aggression, anxiety, depression, somatization, and internalizing symptoms in boys, using the Behavioral Assessment Scale for Children (BASC-2). In girls, a decrease in many symptoms at age 7 years was observed, although none were significant (Harley et al., 2013). In contrast, Braun et al. reported that prenatal BPA urinary concentrations were linked to a decrease in hyperactivity in boys and increases in anxiety, depression, hyperactivity and externalizing symptoms in girls at ages 2–3 years using the BASC (Braun et al., 2011b, 2009). Results for childhood BPA exposure have also been mixed (Braun et al., 2011b; Braun et al., 2009; Harley et al., 2013; Perera et al., 2012). Further, Maserejian et al. were unable to attribute changes in child behavior specifically to the BPA in dental composites (Maserejian et al., 2012).

In the present study, we followed-up on our prior analyses (Perera et al., 2012) and examined the association between prenatal and early childhood BPA urinary concentrations on

neurobehavioral symptoms in children at ages 7–9 years. We hypothesized that we would continue to observe the sex-specific relationships previously reported (Harley et al., 2013; Perera et al., 2012).

2. Methods

Sample selection. A complete description of the NYC cohort and study design appears elsewhere (Perera et al., 2003, 2006). Briefly, subjects included mothers and children participating in the Columbia Center for Children's Environmental Health (CCCEH) prospective cohort study. Between 1998 and 2006, 727 pregnant women residing in Washington Heights, Harlem and the South Bronx were recruited in prenatal clinics to participate in the study. To avoid potential confounding, only women ages 18–35 years, non-smokers, non-users of other tobacco products and/or illicit drugs, those generally in good health (free of known diabetes, hypertension and HIV) and those who initiated prenatal care by 20 weeks of pregnancy were included in the study. In-person postnatal questionnaires were given when the child was 6 months and annually thereafter with developmental questionnaires administered every 1–2 years. Informed consent was provided for children by the mothers until age 7 at which point the children gave assent to participate. The Institutional Review Boards of the Columbia University Medical Center and of the Centers for Disease Control and Prevention (CDC) approved this study. Collection of urine during pregnancy began in 1999 (Hoepner et al., 2013), the year after initial recruitment and data collection began. 370 mothers provided urine samples during pregnancy for measurement of BPA. 271 of their children were followed through ages 7–9 when CBCL data were obtained.

BPA measures. Spot urine samples were collected from the mother during the third trimester of pregnancy and from the children at ages 3 and 5 years. After collection, the samples were sent to the CCCEH laboratory, inventoried, stored at -80°C and subsequently shipped to the CDC for analysis. Total (free plus conjugated) urinary concentrations of BPA were measured using online solid-phase extraction coupled with high-performance liquid chromatography-isotope dilution-tandem mass spectrometry with peak focusing as described before (Ye et al., 2005) with appropriate quality control samples in each run. The limit of detection (LOD) was $0.4\ \mu\text{g/L}$. Concentrations below the LOD were given a value of LOD/2 for statistical analysis. Specific gravity (SG) measurements were obtained using a handheld refractometer (Urine-Specific-Gravity-Refractometer-PAL-10-S-P14643C0; TAGO USA, Inc., Bellevue, WA). To adjust for urinary dilution, we used the formula: $\text{BPAC} = \text{BPA} \times [(\text{mean SG} - 1)/(\text{individual SG} - 1)]$ where BPAC is the SG-corrected BPA concentration ($\mu\text{g/L}$), BPA is the measured BPA concentration ($\mu\text{g/L}$), SG is the specific gravity of the urine sample, and mean SG is the mean SG in the study population calculated separately for maternal, child age 3 and child age 5 samples (Hauser et al., 2004).

Behavioral outcomes. When children reached ages 7 and 9 years, research workers trained in neurodevelopmental testing oversaw the completion of the CBCL by the mothers, providing guidance as needed. The 113-item CBCL was available in both English and Spanish. The CBCL consists of eight syndrome scales (anxious/depressed, withdrawn/depressed, somatic complaints, social problems, thought problems, attention problems, rule-breaking behavior and aggressive behavior) and two composite scales, internalizing problems (sum of scores on the anxious/depressed, withdrawn/depressed and somatic complaints scales) and externalizing problems (sum of scores on the rule-breaking behavior and aggressive behavior scales). The responses to each question are given a numeric value and summed to yield a raw score

measuring the degree of problems on the specified scale. The CBCL has been shown to have high validity and reliability (Achenbach and Rescorla, 2001).

Statistical analysis. For all analyses, both maternal and childhood BPA concentrations (SG adjusted) were dichotomized at the upper tertile (high: upper tertile vs. low: other two tertiles). This approach was supported by the calculated residuals for each tertile of SG adjusted BPA (collected during pregnancy and early childhood) on internalizing and externalizing problems in boys and girls separately. In general, we observed that the median of the residuals within the highest tertile differed from the medians of the lower two tertiles, which were approximately equal. Although several samples are preferable to properly characterize BPA exposure (Braun et al., 2012; Meeker et al., 2013; Quiros-Alcala et al., 2013), a single spot urine sample can adequately classify individuals into the highest BPA tertile vs. the lower two tertiles of BPA exposure (Mahalingaiah et al., 2008), despite low intra-class correlations for concentrations from multiple samples (Braun et al., 2011a) and high daily variance (Ye et al., 2011). For the postnatal BPA measure, age 5 year samples were used unless a child was missing this sample in which case the age 3 year sample was used. Out of the $n=250$ children included in the analysis, 22 had a 3 year urine sample only, 65 had a 5 year sample only and 163 had both a 3 year and 5 year urine sample.

CBCL scores at age 7 served as the outcome except in cases where this score was missing and age 9 CBCL data were used. In the present study, 22 had CBCL data at age 7 only, 31 had CBCL data at age 9 only and 197 had CBCL data at both ages 7 and 9. Each of the individual syndrome and composite scales raw scores was analyzed as a continuous variable. Because the raw scores of each syndrome and composite scale are count data and have right-skewed distributions, Poisson regression models were used to analyze the relationship between scores and BPA urinary concentrations. Regression models were analyzed for both sexes combined and also stratified by sex.

We further examined the BPA \times sex interaction on the eight CBCL syndrome scales and the two composite scales using Poisson regression, with a significant interaction term defined as having a regression coefficient that differed from 0 with a p -value ≤ 0.05 . The interaction term is coded such that a negative coefficient means that girls in the highest tertile of BPA concentration compared to girls in the lower two tertiles experienced fewer symptoms than boys in the highest tertile compared to boys in the lower two tertiles. All regression coefficient estimates and p -values were generated using SAS (version 9.3 SAS Institute Inc., Cary, NC). Although our analyses involved multiple tests, we did not perform the Bonferroni adjustment in order to reduce the possibility of making a type II error (Rothman, 1990). Rather, our focus was on the sex-specific patterns of the observed relationships and their consistency with those seen in our cohort children at an earlier age (Perera et al., 2012).

In our main analyses with prenatal BPA, we stratified on child sex and adjusted for potential confounding variables including known or suspected risk factors for the exposure or outcome based on the literature and our previous results. These included: ethnicity, gestational age of the child (based on medical record data), mother's intelligence (measured by the Test of Nonverbal Intelligence 3rd Edition, TONI-3 (Brown et al., 1997)), maternal education (completion of high school prior to birth of child), maternal demoralization (measured by the Psychiatric Epidemiology Research Instrument-Demoralization (PERI-D) scale) (Dohrenwend et al., 1978), child age (in months) at CBCL testing, quality of the child's home environment at 3 years of age (measured by the HOME (Home Observation for Measurement of the Environment) Inventory) (Bradley, 1994), prenatal exposure to environmental tobacco smoke (ETS), SG-adjusted postnatal BPA concentration

(dichotomized at the upper tertile), and SG-adjusted mono-*n*-butyl phthalate (MnBP) in maternal urine collected during the third trimester of pregnancy (Adibi et al., 2008). Similar analyses with postnatal BPA urinary concentrations as the main effect were conducted, adjusting for prenatal BPA urinary concentrations. As in our previous studies (Rundle et al., 2012), to account for potential selection bias, we also conducted analyses with inverse probability weighting (IPW).

Finally, we imputed missing values for covariates except for prenatal MnBP or postnatal BPA concentrations using a multiple imputation method with five imputed datasets, as described (Rubin, 1987). The results reported are based on multiple imputation. In no case were data lacking for more than 8 percent of subjects. We note that there were no observed differences in results before and after conducting imputation.

3. Results

The present study sample included 250 children (135 girls and 115 boys) consisting of those with complete data on CBCL and pre and postnatal BPA urinary concentrations (Table 1). The children included in this analysis did not significantly differ in terms of exposures and maternal/child characteristics from those who were not included due to missing CBCL ($N=99$) or covariate data for those covariates not imputed ($N=21$).

A total of 245/250 (98 percent) prenatal samples and 245/250 (98 percent) postnatal samples (at age 3 or 5 years) had detectable BPA concentrations. The ranges and percentile distribution of prenatal BPA urinary concentrations and child urinary BPA concentrations at age 3 or 5 are shown in Table 2. A wide range of prenatal and postnatal BPA concentrations was observed within our cohort with greater geometric means seen during childhood. Prenatal and childhood BPA concentrations were not significantly correlated ($r = -0.01$, $p = 0.82$).

As shown in Table 3, compared to girls in the lower two tertiles of prenatal urinary BPA concentrations, those in the upper tertile experienced significantly fewer internalizing symptoms. Specifically, girls with high prenatal BPA concentrations experienced a 0.17 point decrease in internalizing CBCL score ($p = 0.04$) and a 0.40 point decrease in withdrawn/depressed score ($p = 0.03$) as compared to girls in the lower two tertiles of BPA concentration. Conversely, boys born to mothers in the highest prenatal BPA concentration tertile experienced higher internalizing ($\beta = 0.41$, $p < 0.0001$) and externalizing ($\beta = 0.40$, $p < 0.0001$) scores as compared to those in the lower concentration tertiles. A similar pattern was seen on the three individual syndrome scale scores that contribute to the internalizing composite score: anxious/depressed ($\beta = 0.48$, $p = 0.0002$), withdrawn/depressed ($\beta = 0.36$, $p = 0.05$) and somatic complaints ($\beta = 0.32$, $p = 0.04$). Associations with scores on the individual syndrome scales that contribute to the externalizing composite score, rule-breaking ($\beta = 0.40$, $p = 0.005$) and aggressive behaviors ($\beta = 0.39$, $p < 0.0001$), were also positive and statistically significant (Table 3). Significant and borderline significant interactions were observed between prenatal BPA urinary concentrations and sex for both the internalizing ($\beta = -0.36$, $p = 0.002$) and externalizing composite scores ($\beta = -0.20$, $p = 0.06$). All interactions (except thought problems) were in the negative direction (Table 3). In separate models not adjusting for postnatal BPA, the associations remained significant.

In addition, we assessed the associations of postnatal BPA exposure on the CBCL outcomes, adjusting for prenatal exposure. Significant results were seen among girls and boys for internalizing and externalizing problems composite scores, and the anxiety/depression, rule-breaking behavior and aggressive behavior subscores, in addition to significant interactions between sex and BPA

Table 1
Characteristics of children included ($N=250$) in the present study.^a

Variable	Mean \pm SD or %
Prenatal BPA urinary concentration ($\mu\text{g/L}$) ^b	3.2 \pm 4.7
Postnatal BPA urinary concentration ($\mu\text{g/L}$) ^{b,c}	5.2 \pm 6.5
Prenatal mono-n-butyl phthalate urinary concentration ($\mu\text{g/L}$) ^b	64.5 \pm 95.4
Age at assessment (months)	85.6 \pm 5.3
Percent with prenatal ETS exposure	31.6
Percent female	54.0
Percent $>=$ high school education	61.2
Percent African American	35.2
Gestational age at birth (weeks)	39.3 \pm 1.3
Maternal TONI score	19.9 \pm 8.4
HOME inventory	39.9 \pm 5.6
Maternal demoralization score	1.1 \pm 0.6

Values are mean \pm SD or percent.

^a A total of 370 children had available information on prenatal BPA concentration. Some subjects were not included in the analysis due to missing CBCL measures ($n=99$), or missing information on postnatal BPA concentration or prenatal MnBP concentration ($n=21$).

^b BPA and Phthalate concentrations adjusted for specific gravity.

^c Postnatal BPA concentrations were measured in child spot urine samples at ages 3 and 5 years. For this analysis, the sample taken at age 5 was used unless it was missing, in which case, the age 3 sample was used.

urinary concentrations for each of these domains (Table 4). Girls in the highest tertile of BPA concentration experienced a 0.30 point increase in score on the internalizing symptoms composite score ($p=0.0002$) and a 0.33 point increase in score on the externalizing problems score ($p<0.0001$) as compared to girls in the lower two tertiles of BPA concentration. In contrast, for boys negative associations between high postnatal BPA concentration and internalizing ($\beta=-0.29$, $p=0.002$) and externalizing ($\beta=-0.37$, $p<0.0001$) composite scores were observed. The interaction coefficient between sex and postnatal BPA concentration for each of these scores was significant (Table 4). We note that in separate models not adjusting for prenatal BPA, these associations remained significant.

After accounting for selection bias based on the IPW method, we found that the associations between prenatal BPA concentration and higher externalizing behaviors composite score and

subscores in boys remained consistent, but associations between prenatal BPA and fewer behavior problems among the girls were no longer statistically significant. On the contrary, the associations between postnatal BPA and more behavior problems including both internalizing and externalizing composite scores remained statistically significant among the girls after conducting IPW, while the associations between postnatal BPA and fewer behavior problems among boys were no longer significant (with the exception of fewer rule breaking behavior problems among boys, which remained significant). These results are presented in Supplemental Tables 1 and 2.

4. Discussion

Consistent with our prior report at ages 3–5 years (Perera et al., 2012), the observed associations of prenatal BPA urinary concentrations on CBCL scores in our NYC cohort of Dominican and African-American children at ages 7–9 years differed between girls and boys. In girls, higher prenatal BPA urinary concentrations were associated with lower behavioral symptom scores (significant for withdrawn/depressed symptoms and internalizing problems) and with increased symptom scores in boys (significant for anxious/depressed symptoms, somatic complaints, thought problems, rule-breaking behavior, aggressive behavior, and total internalizing and externalizing behaviors). These findings for internalizing behaviors are similar to those reported in a primarily Hispanic cohort in Salinas Valley, California (Harley et al., 2013) but differ from those in a largely white cohort in Cincinnati, Ohio (Braun et al., 2011b; Braun et al., 2009). The discrepancy may be due to differences in co-exposures, genetic factors, and social stressors experienced by the mothers and children in the respective cohorts. Further, we found that postnatal BPA concentration had the opposite effects such that postnatal BPA concentration was associated with more behavior problems among girls but fewer among boys.

The CBCL is a screening test and the outcomes are not clinical diagnoses. Nevertheless, our overall findings of a similar pattern at ages 3–5 years (Perera et al., 2012) and 7–9 years suggest that BPA exposure may be contributing to changes in behavioral problems in children. Other studies have linked behavioral problems such as

Table 2
Prenatal and postnatal BPA urinary concentrations^a ($N=250$).

	Percentile					Maximum	Mean	Geometric mean
	5th	25th	50th	75th	95th			
Prenatal BPA^a								
No specific gravity adjustment ($\mu\text{g/L}$)								
All children	< LOD	1.1	1.9	3.4	9.1	47.2	3.2	1.9
Girls	< LOD	1.0	1.9	3.3	10.7	47.2	3.3	1.8
Boys	< LOD	1.3	1.9	3.6	8.9	30.4	3.1	2.0
Specific gravity adjusted ($\mu\text{g/L}$)								
All children	< LOD	1.2	2.0	3.4	8.1	50.7	3.3	2.1
Girls	< LOD	1.2	2.0	3.4	12.5	50.7	3.4	2.1
Boys	< LOD	1.2	2.0	3.4	7.1	39.0	3.2	2.1
Postnatal BPA^a								
No specific gravity adjustment ($\mu\text{g/L}$)								
All children	0.5	1.7	3.0	6.3	14.8	54.1	5.2	3.2
Girls	0.5	1.6	2.9	6.3	22.2	54.1	5.4	3.0
Boys	0.8	2.2	3.1	6.1	14.5	27.7	4.9	3.4
Specific gravity adjusted ($\mu\text{g/L}$)								
All children	0.8	1.8	3.1	6.1	30.4	404.0	8.1	3.6
Girls	0.7	1.7	3.1	7.1	33.5	404.0	10.4	3.7
Boys	1.0	1.9	3.1	5.7	19.1	33.3	5.4	3.4

^a Limit of detection (LOD)=0.4 $\mu\text{g/L}$. Prenatal BPA concentrations were measured in maternal spot urine samples taken during the third trimester of pregnancy. Postnatal BPA concentrations were measured in child spot urine samples at ages 3 and 5 years. For this analysis, the sample taken at age 5 was used unless it was missing, in which case, the age 3 sample was used.

Table 3Associations between dichotomized prenatal BPA concentrations^a and CBCL scores at ages 7–9 years. ^{b,c}

	Girls (N=135)		Boys (N=115)		Interaction (N=250)	
	Estimate (95% CI)	p-Value	Estimate (95% CI)	p-Value	Estimate (95% CI)	p-Value
Composite scores						
Internalizing problems	-0.17 (-0.33, -0.01)	0.04*	0.41 (0.24,0.58)	< 0.0001**	-0.36 (-0.59, -0.14)	0.002**
Externalizing problems	-0.04 (-0.20, 0.12)	0.63	0.40 (0.24,0.56)	< 0.0001**	-0.20 (-0.42, 0.01)	0.06
Individual scores						
Anxious/depressed	-0.01 (-0.20, 0.23)	0.94	0.48 (0.23,0.72)	0.0002**	-0.26 (-0.59, 0.06)	0.11
Withdrawn/depressed	-0.40 (-0.75, -0.04)	0.03*	0.36 (0, 0.72)	0.05*	-0.49 (-0.98, 0.01)	0.05*
Somatic complaints	-0.28 (-0.59, 0.03)	0.08	0.32 (0.01,0.62)	0.04*	-0.45 (-0.86, -0.04)	0.03*
Social problems	-0.24 (-0.49, 0.02)	0.07	0.17 (-0.08, 0.43)	0.18	-0.24 (-0.59, 0.10)	0.17
Thought problems	0.33 (-0.03, 0.68)	0.08	0.41 (0.08, 0.75)	0.01**	0.13 (-0.33, 0.59)	0.58
Attention problems	-0.09 (-0.33, 0.14)	0.44	0.17 (-0.03, 0.38)	0.10	-0.10 (-0.40, 0.20)	0.52
Rule breaking behavior	-0.12 (-0.44, 0.19)	0.45	0.40 (0.10, 0.68)	0.005**	-0.38 (-0.78, 0.02)	0.06
Aggressive behavior	-0.01 (-0.20, 0.18)	0.92	0.39 (0.2, 0.58)	< 0.0001**	-0.13 (-0.39, 0.13)	0.31

^a The prenatal BPA concentration was dichotomized at the upper tertile.^b For children missing CBCL scores at age 7, CBCL scores from age 9 were used.^c Models adjusted for ethnicity, gestational age, maternal intelligence, maternal education, maternal demoralization, child age at CBCL testing, quality of the child's home environment, prenatal ETS exposure, specific-gravity (SG) adjusted postnatal BPA concentration at age 3 or 5, and maternal mono-*n*-butyl phthalate concentration collected during the third trimester.* *p*-Value ≤ 0.05.** *p*-Value ≤ 0.01.

internalizing symptoms and attention problems with adverse learning outcomes and poor school performance (Bub et al., 2007; Grimm et al., 2010), both of which adversely impact future economic and emotional well-being.

Regarding possible epigenetic mechanisms, a recent experimental study suggests that prenatal BPA exposure alters expression of genes encoding the estrogen receptors, ER- α , ER- β and estrogen-related receptor- γ , with corresponding changes in mRNA levels of DNA methyltransferases DNMT1 and DNMT3A and methylation of the ER- α gene in the cortex and hypothalamus of male and female mice, respectively. The mice exposed to high BPA doses experienced significant changes in social behavior, anxiety and aggression (Kundakovic et al., 2013). Additional studies have shown that rodents exposed to BPA prenatally had increased levels of anxiety (Cox et al., 2010; Luo et al., 2014; Patisaul and Bateman, 2008; Ryan and Vandenberg, 2006; Tian et al., 2010; Xu et al., 2014; Xu et al., 2011; Yu et al., 2011), aggression (Patisaul and

Bateman, 2008), and hyperactivity (although the sex-specificity of these associations was not consistent between studies) (Ishido et al., 2004; Ishido et al., 2007; Masuo et al., 2004; Xu et al., 2007), and a loss of sexual dimorphism for spatial learning and memory outcomes (Ryan and Vandenberg, 2006; Xu et al., 2007; Xu et al., 2011). Taken together, these studies suggest that epigenetic mechanisms may underlie the effects of BPA on behavior; however, data are lacking in humans.

In the present study, the direction of association observed differed between boys and girls and depended on the timing of the exposure (prenatal exposure vs. early childhood exposure). In terms of the sex-specific differences observed, our findings are consistent with prior data suggesting a greater vulnerability of the male brain during the prenatal period (Harley et al., 2013; Perera et al., 2012). A number of studies have found evidence of stronger associations among boys on outcomes such as behavior problems and reduced IQ with increasing exposure to environmental

Table 4Associations between dichotomized postnatal BPA concentrations^a and CBCL scores at ages 7–9 years. ^{b,c}

	Girls (N=135)		Boys (N=115)		Interaction (N=250)	
	Estimate (95% CI)	p-Value	Estimate (95% CI)	p-Value	Estimate (95% CI)	p-Value
Composite scores						
Internalizing problems	0.30 (0.14,0.45)	0.0002**	-0.29 (-0.47, -0.11)	0.002**	0.49 (0.26, 0.71)	< 0.0001**
Externalizing problems	0.33 (0.17, 0.5)	< 0.0001**	-0.37 (-0.54, -0.20)	< 0.0001**	0.71 (0.49, 0.93)	< 0.0001**
Individual scores						
Anxious/depressed	0.35 (0.12, 0.58)	0.003**	-0.33 (-0.60, -0.06)	0.02*	0.59 (0.25, 0.92)	0.0006**
Withdrawn/depressed	0.29 (-0.03, 0.62)	0.08	-0.26 (-0.64, 0.13)	0.20	0.40 (-0.07, 0.87)	0.10
Somatic complaints	0.19 (-0.09, 0.48)	0.19	-0.29 (-0.61, 0.03)	0.08	0.40 (0, 0.81)	0.05*
Social problems	0.14 (-0.11, 0.39)	0.26	-0.0007 (-0.25, 0.25)	1.0	0.10 (-0.24, 0.44)	0.56
Thought problems	0.18 (-0.19, 0.54)	0.34	-0.07 (-0.40, 0.26)	0.67	0.30 (-0.16, 0.77)	0.20
Attention problems	-0.06 (-0.30, 0.18)	0.62	-0.16 (-0.37, 0.05)	0.13	0.20 (-0.10, 0.50)	0.18
Rule breaking behavior	0.46 (0.16, 0.76)	0.003**	-0.47 (-0.77, -0.17)	0.002**	0.85 (0.44, 1.26)	< 0.0001**
Aggressive behavior	0.29 (0.10, 0.48)	0.003**	-0.32 (-0.53, -0.12)	0.002**	0.66 (0.39, 0.92)	< 0.0001**

^a The postnatal BPA (age 3 or 5) concentration was dichotomized at the upper tertile.^b For children missing CBCL scores at age 7, CBCL scores from age 9 were used.^c Models adjusted for ethnicity, gestational age, maternal intelligence, maternal education, maternal demoralization, child age at CBCL testing, quality of the child's home environment, prenatal ETS exposure, specific-gravity (SG) adjusted prenatal BPA concentration collected during the third trimester, maternal SG-adjusted mono-*n*-butyl phthalate concentration.* *p*-Value ≤ 0.05.** *p*-Value ≤ 0.01.

toxicants during the prenatal period including BPA (Harley et al., 2013), lead (Jedrychowski et al., 2009), and chlorpyrifos (Horton et al., 2012). However, the sex-dependent nature of these chemicals likely depends on the nature and timing of exposure. For example, our current analysis suggests that boys experienced increased behavioral symptoms in response to prenatal BPA exposure, but decreased behavioral symptoms in response to early childhood BPA exposure. A reversal of patterns was also seen among girls, but with increased scores seen in response to early childhood BPA exposure. A similar direction and reversal of trends were seen in an earlier study in the CHAMACOS cohort (Harley et al., 2013). In their study, no significant associations were seen between prenatal BPA concentration and behavioral symptoms in girls, but significant associations were present when assessing early childhood BPA concentration. Boys experienced increased symptom scores in response to prenatal BPA concentration, though, contrary to our results, boys also experienced increased symptom scores in response to early childhood BPA concentration. However, the early childhood results in boys were only seen among the teacher-reported behavioral symptoms (Harley et al., 2013). In studies by Braun et al., sex-specific associations with childhood behavioral outcomes were seen only in response to prenatal BPA concentration and not childhood BPA concentration with girls experiencing increases in anxiety, depression, hyperactivity, and externalizing problems and boys experiencing decreases in hyperactivity (Braun et al., 2011b, 2009). The finding that results differ between boys and girls is plausible given the endocrine-disrupting nature of BPA, though the reason behind the discrepancies between prenatal and early childhood exposure are unclear.

The present study has a number of strengths including the prospective design. At 7–9 years of age we found results consistent with our prior findings at age 3–5 years. The large number of variables collected from medical records, questionnaires and biological samples across the span of our study allowed us to control for multiple potential confounders at different time points.

Study limitations include the limited sample size and the assessment of BPA exposure based on a single measurement collected during pregnancy. Because BPA has a short half-life and exposures are likely episodic in nature, the single spot urine sample collected may not adequately describe a subject's chronic exposure. However, as noted, the urinary concentration from a single urine sample has moderate sensitivity in estimating an individual's BPA tertile categorization (Mahalingaiah et al., 2008), and by using a dichotomized variable, we aimed to avoid measurement error. Furthermore, given the potential for noise in these measurements, we would expect the result to be biased towards the null. In addition, to maximize our sample size, for the postnatal BPA variable we used the BPA concentration obtained at age 5 or, if that were missing, the BPA concentration at age 3. Although known factors that may confound the association between BPA exposure and behavioral outcomes were adjusted for, it is possible that residual confounding or additional bias remains. Generalizability was reduced by the ethnicity of our cohort (African-American and Dominican). However, these ethnic groups make up a large and growing fraction of the U.S. population of women of reproductive age. Finally, we cannot rule out the possibility of selection bias; however, we used IPW to evaluate the impact of this potential bias. Using IPW, we found that prenatal BPA exposure was associated with significantly more problems in boys and postnatal exposure was associated with significantly more problems in girls. We, therefore, have more confidence in these findings and believe that they are less likely to be a function of a biased sample.

5. Conclusion

These results suggest that prenatal and early childhood BPA exposure may affect behavioral outcomes in a sex-specific and timing-specific manner among inner-city children. They raise concern about the pervasiveness of this chemical and its potential effects in children from early-life exposures. In our study, the direction of associations seen between BPA concentration and behavioral symptom scores depended on the timing of exposure and differed for boys and girls. The mechanisms of BPA in relation to human health are complex and multi-factorial. Further research in both humans and animal models is recommended to further elucidate the effects of BPA on the brain development and behavioral outcomes.

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Human subjects research

All activities were approved by the Institutional Review Boards at the Columbia University Medical Center under human subjects protocol number AAAA-6110, and by the Centers for Disease Control and Prevention.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.envres.2015.01.014>.

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