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Endocrine Disruptors and Childhood Social Impairment

Amir Miodovnik[a], **Stephanie M. Engel**[a], **Chenbo Zhu**[a], **Xiaoyun Ye**[c], **Latha V. Soorya**[b], **Manori J. Silva**[c], **Antonia M. Calafat**[c], and **Mary S. Wolff**[a]

[a]Department of Preventive Medicine, Mount Sinai School of Medicine, New York, NY, United States

[b]Department of Psychiatry, Mount Sinai School of Medicine, New York, NY, United States

[c]National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA, United States

Abstract

Prenatal exposure to endocrine disruptors has the potential to impact early brain development. Neurodevelopmental toxicity *in utero* may manifest as psychosocial deficits later in childhood. This study investigates prenatal exposure to two ubiquitous endocrine disruptors, the phthalate esters and bisphenol A (BPA), and social behavior in a sample of adolescent inner-city children. Third trimester urines of women enrolled in the Mount Sinai Children's Environmental Health Study between 1998 and 2002 (n = 404) were analyzed for phthalate metabolites and BPA. Mother-child pairs were asked to return for a follow-up assessment when the child was between the ages of 7 to 9 years. At this visit, mothers completed the Social Responsiveness Scale (SRS) (n = 137), a quantitative scale for measuring the severity of social impairment related to Autistic Spectrum Disorders (ASD) in the general population. In adjusted general linear models increasing log-transformed low molecular weight phthalate (LMW) metabolite concentrations were associated with greater social deficits (β = 1.53, 95% CI 0.25-2.8). Among the subscales, LMWP were also associated with poorer Social Cognition (β = 1.40, 95% CI 0.1-2.7); Social Communication (β = 1.86, 95% CI 0.5-3.2) and Social Awareness (β = 1.25, 95% CI 0.1-2.4), but not for Autistic Mannerisms or Social Motivation. No significant association with BPA was found (β = 1.18, 95% CI: -0.75, 3.11). Prenatal phthalate exposure was associated with childhood social impairment in a multiethnic urban population. Even mild degrees of impaired social functioning in otherwise healthy individuals can have very important adverse effects over a child's lifetime. These results extend our previous finding of atypical neonatal and early childhood behaviors in relation to prenatal phthalate exposure.

Keywords

Endocrine Disruptors; Phthalates; BPA; SRS; Autism Spectrum Disorder; Environmental Exposure

Corresponding Author: Dr. Amir Miodovnik, Department of Preventive Medicine, Mount Sinai School of Medicine, One Gustave L. Levy Place, Box 1057, New York NY 10029, USA, Phone: 212-824-7038, Fax: 212-966-0407, amir.miodovnik@mssm.edu.

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Introduction

Phthalates and bisphenol A (BPA) are high volume synthetic chemicals with annual production in the billions of pounds worldwide. They are commonly found in consumer products and human exposure arises from inhalation, ingestion, and dermal contact with such products. The Centers for Disease Control and Prevention (CDC) as part of the National Health and Nutrition Examination Survey (NHANES) has consistently found urinary concentrations of phthalates and BPA in a representative sample of the U.S. general population aged 6 years and older (CDC 2009). The heightened concern surrounding exposure to these ubiquitous chemicals relates to their classification as endocrine disruptors (EDs), hormonally-active compounds linked to reproductive toxicity in both animals and humans (CERHR 2007; NRC 2008). High molecular weight (HMW) phthalates such as di(2-ethylhexyl) phthalate (DEHP) are plasticizers found in polyvinyl chloride (PVC) plastics, some food packaging, intravenous tubing, building materials, and children's toys. Low molecular weight (LMW) phthalates, including diethyl phthalate (DEP) and dibutyl phthalate (DBP), are common constituents of personal care products (e.g., cosmetics, shampoos and nail polish), fragrances, and in the coating of certain medications (NRC 2008). BPA is a monomer used in the manufacture of polycarbonate re-usable bottles among other consumables, in epoxy resins that coat and protect the insides of metal food containers, and in dental composites and sealants (CERHR 2007). Based on NHANES data, BPA and phthalates have been detected in over 90% of the US general population (CDC 2009). Additional biomonitoring studies have found detectable levels of these industrial compounds in a wide range of body tissues including urine, blood, placenta, amniotic fluid and breast milk (CERHR 2007; NRC 2008).

Phthalates exhibit anti-androgenic or weakly estrogenic activity while BPA acts as a weak estrogen; both compounds appear to adversely impact thyroid hormone regulation (Crofton 2008; Boas et al. 2010). The neuroendocrine actions of these EDs may interfere with hormone-sensitive periods of neural development including neuronal differentiation, growth, and synapse formation and subsequent behavior (Colborn 2004). Prenatal BPA exposure in rodent studies is associated with aggressive behavior, memory impairment, increased anxiety, and hyperactivity (Kawai et al., 2003; Miyagawa et al., 2007; Tian et al., 2010). These effects may underlie maladaptive behaviors in humans as well, although epidemiological data is limited. In our own cohort, we have observed associations between higher maternal phthalate exposure and more atypical neonatal behaviors, specifically poorer scores for the Orientation and Motor scales and overall Quality of Alertness, as well as more externalizing behavior problems (i.e., hyperactivity, aggressiveness) and poorer executive functioning in later childhood (Engel et al., 2009; Engel et al., 2010).

Social responsiveness is based in large part on how the brain processes and responds to external social cues (Adolphs 2001). Some pediatric clinical disorders that commonly present with impaired reciprocal social behavior include ASD, Attention-Deficit Hyperactivity Disorder (ADHD), Oppositional Defiant Disorder (ODD) and learning disabilities (Reiersen et al., 2007; Friedman et al., 2003; Carpenter et al., 2009; Bhaumik et al., 1997). Subclinical forms of social impairment, often referred to as autistic-like traits, appear to extend into the general population (Constnantino and Todd 2003; Hoekstra et al., 2007; Ronald et al., 2005). The Social Responsiveness Scale (SRS) is the first widely used quantitative method of identifying autistic traits and problems with social-relational skills related to ASDs in the general population (Constantino and Gruber 2005). Impaired social functioning and autistic traits may serve as markers of maladaptive development associated with early environmental toxicant exposures. We hypothesize that prenatal environmental toxicants such as phthalates and BPA may contribute to some forms of childhood social impairment.

Materials and Methods

The Mount Sinai Children's Environmental Health study is a prospective multiethnic cohort of primiparous women who delivered at Mount Sinai Hospital between May 1998 and July 2002 (Berkowitz et al., 2003; Berkowitz et al., 2004). Of the 479 mothers recruited, seventy-five were excluded for medical complications ($n = 3$), infant or fetal demise ($n = 2$), very premature births (delivery at < 32 completed weeks or < 1,500 g) ($n = 5$), miscarriage ($n = 1$), delivery of an infant with genetic abnormalities or malformations ($n = 5$), inability to collect biologic specimens before birth ($n = 12$), change of hospital or residence outside New York City ($n = 28$), or loss to follow-up or refusal to continue to participate ($n = 19$) (Wolff et al., 2008) leaving 404 for whom birth data were available. Recruitment occurred through a prenatal care center serving the predominantly minority East Harlem population and from one of two private practices on the Upper East Side of Manhattan. Participants completed a questionnaire regarding sociodemographic characteristics, medical history, and lifestyle factors during the third trimester of pregnancy. Women were invited to return for three follow-up visits when their children were between 4 and 9 years of age. At the last childhood evaluation, which occurred between the ages of 7 to 9, mothers completed the Social Responsiveness Scale (SRS) (n = 137), a 65-item caregiver/educator rating scale of social behaviors characteristic of autism spectrum and related disorders. The study was approved by the Institutional Review Board of Mount Sinai School of Medicine; participants provided written informed consent before the study.

The SRS is a well-validated quantitative scale for detecting and measuring the severity of autistic behavior. Each SRS item rates the frequency of a particular behavior using a 4-point Likert scale (0 to 3 points for each item), with higher scores indicating a higher degree of autistic symptoms (Constantino and Gruber 2005). The SRS generates a clinically-relevant standardized total score (total *T*-score); the subscale *T*-scores include Social Awareness, Social Cognition, Social Communication, Social Motivation, and Autistic Mannerisms provide more in-depth analysis of the total score when the instrument is used in clinical settings, but they are not diagnostic. The Social Awareness subdomain measures one's ability to discern social cues and sensory aspects of social interactions. The Social Cognition subdomain measures deficits in interpreting social cues and other cognitive functions. The Social Communication subdomain captures deficits in pragmatic and expressive communication, which are the primary deficits seen in high-functioning children with ASDs. The cumulative deficits in these subdomains may reflect higher level socialization difficulties and impairments in both engaging in and interpreting fast-paced social interactions. In contrast, the more classically autistic behaviors captured by the Social Motivation (e.g. avoiding social interactions) and Autistic Mannerisms (e.g. highly restricted interests, stereotypical motor activity) subdomains reflect more severe ASD symptoms.

T-Scores have a mean of 50 and a standard deviation of 10 and have been calculated separately for males and females. Higher total *T*-scores on the SRS indicate greater severity of social impairment in the autism spectrum. *T*-scores between 60 and 75 indicate deficiencies in social behavior that are clinically significant and may interfere with everyday social interactions; scores greater than 75 are strongly associated with a clinical diagnosis of Autistic Disorder, Asperger's Disorder, or more severe cases of Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS) and severe interference with everyday social interactions (Constantino and Gruber 2005). The standardization sample showed no evidence of an age effect and the various ages groups differed by no more than 0.2 standard deviations from the overall group mean. The SRS has good retest temporal stability, parent-parent and parent-teacher interrater agreement and discriminate and concurrent validity (Constantino and Gruber 2005). The parent-report SRS exhibits substantial agreement with

the Autism Diagnostic Interview-Revised (ADI-R), a widely recognized gold standard for establishing a research diagnosis of autistic disorder (Constantino et al., 2003).

Maternal spot urine samples were collected during pregnancy between 25 and 40 weeks (mean of 31.2 weeks) gestation and analyzed by the CDC for 10 individual phthalate metabolites and the phenolic compound, BPA as described in detail elsewhere (Kato et al., 2005; Ye et al., 2005). Urinary concentrations of the biomarkers were examined both as micrograms per liter and corrected for urine dilution as micrograms per gram creatinine (μg/gC). To limit the influence of multiple comparisons on our findings, phthalate metabolites were grouped into micromolar sums (μmol/L) of high-molecular-weight (> 250 Da) monoester metabolites (HMW) and low molecular-weight (< 250 Da) monoester metabolites (LMW). The phthalate metabolites within each grouping represent similar molecular structure, biological activity, and sources of exposure as the parent diester (Wolff et al., 2008). The individual phthalate metabolites and the high and low molecular weight micromolar sums were used as continuous variables in multivariable analysis. Limits of detection (LOD) were in the low microgram per liter (μg/L) range. Phthalate metabolites (except for monomethyl phthalate, MMP) and BPA were detectable in over 90% of our subjects; those below the LOD were assigned the value of LOD divided by the square root of 2 (Hornung and Reed 1990). Continuous biomarker values and creatinine were natural log transformed (ln) to produce more normal distributions. We accounted for urine dilution by including log-creatinine in models where biomarkers were continuous log-transformed variables.

Data were analyzed using SAS version 9.2 (Cary, NC). Phthalate metabolites and BPA urinary concentrations were transformed using the natural log to approximate a normal distribution. After examining unadjusted correlations of concentrations of BPA and phthalate metabolites to SRS total score, general linear models (using PROC GLM) were used to analyze relationships between natural log transformed biomarker concentrations and continuous SRS total and subscale scores. The following were considered as potential confounders or covariates: maternal age (continuous variable), maternal IQ, marital status at the time of follow-up (single caretaker versus living with both parents), maternal education (less than high school versus more than a high school), child race (non-Hispanic white, non-Hispanic black, or Hispanic), sex, child IQ, exact age at examination, and urinary creatinine. Backward elimination was used to arrive at the final adjusted models. Covariates were eliminated from the full model if their exclusion caused less than a 10% change in the exposure beta coefficient of the full model for the SRS Total Score. This final parsimonious model was applied to the SRS subscales for continuity and comparability. Maternal age was not associated with SRS score. Maternal IQ and child IQ were negatively associated with total SRS score, but they did not change the main effect of the model. Interactions of prenatal phthalate and phenols exposure with child sex were tested and found to be nonsignificant ($p > 0.10$). In BPA analyses, models were run with and without outliers. Outliers were detected using an approach that detects influential observations according to their effect on predicted values (implemented in SAS via the Difference in Fit Statistic (DFFITS)) and observations that appear to be inconsistent with the rest of the data (implemented in SAS via the studentized deleted residuals (RSTUDENT)). The specific criteria were DFFITS greater than 2, and RSTUDENT greater than the absolute value of 2.

Results

Maternal and infant characteristics of the original cohort have been previously reported (Engel et al., 2007). Slight differences were observed in the characteristics of women who completed the SRS versus the original birth cohort (Table 1). Compared with the original birth cohort (n=404), the women who returned for follow-up (n=137) were more likely to be

single or divorced and had attained a higher level of education; median urinary concentrations of the low and high molecular phthalates metabolites were similar between the cohorts while BPA was slightly lower in the follow up cohort (Table 2). The median LMW phthalate metabolite concentration for our population was 419 μg/L. The median urine BPA concentration for our study population was1.2 μg/L, lower than the NHANES 2003-2004 median level of 2.6 μg/L (Calafat et al., 2008). The LMW phthalate metabolite concentrations for thirty-one (22.6%) children who met the threshold for "Mild to Moderate" (SRS *T*-score of 60 to 74) or "Severe" (SRS *T*-score ≥ 75) social impairment in this population were 460 μg/L (n = 25) and 1260 μg/L (n = 6), respectively (Table 3). Although for phthalates and BPA the median exposure level in the Severe Social Impairment category was the highest, there was a wide range of exposure in this group and few cases of Severe Impairment overall.

In unadjusted models, we found a positive correlation of LMW phthalate (Spearman rho = 0.24. p = 0.005) and BPA level with total SRS (Spearman rho = 0.25, p = 0.004) (Figure 1). Positive correlations reflect poorer SRS scores with increasing BPA and phthalate metabolite concentrations. In multivariable adjusted linear models, we examined the association of continuous urinary concentrations of phthalate molar sums, individual phthalate metabolites and BPA with SRS total *T*-score (Table 3). LMW phthalate concentrations were strongly related to total SRS score and a number of subscales. Each log-unit increase in LMW phthalate metabolite concentration was associated with higher SRS scores. Among the subscales, LMW phthalate metabolite concentrations were positively associated with poorer scores on Social Cognition, Social Communication, and Social Awareness, but not for Social Motivation or Autistic Mannerisms (Table 4). Furthermore, the regression coefficients for total and subscale scores were generally consistent in direction and comparable in precision. Of the individual LMW phthalate metabolites, only MEP was statistically significant, consistent with the major contribution of MEP to the total LMW phthalate sum; however, the effect size was, in general, consistent for monobutyl phthalate (MBP), MEP and MMP. There were no consistent associations of SRS scores with HMW phthalate metabolites. For BPA, regression coefficients were consistent with LMW phthalates in direction, but with a weaker main effect estimate and measured with substantially less precision. Additionally, although there was no significant interaction between creatinine concentration and BPA, the BPA effect appeared to be sensitive to urinary dilution and BPA concentration, suggesting a higher likelihood of exposure misclassification when urines were dilute or exposure values were very low. The large number of participants with BPA concentrations near the LOD and the lower BPA exposure levels in our study population may partly explain the weak overall association we observe for BPA in relation to total SRS score. When influential outliers are excluded from the full BPA model (n = 6), however, the magnitude of the BPA associations for total and subscale scores are quite similar to those for LMW phthalates although the precision is unchanged (Table 4).

Discussion

Our findings add to the growing body of literature examining industrial chemical exposures and developmental neurotoxicity in humans. A few studies have suggested that there may be an association between postnatal phthalate exposure and atypical social functioning in school-aged children. One ecological study found that children 1 to 3 years of age who lived in homes with PVC flooring, an important indoor source of phthalate dust, had higher rates of ASD when followed up 5 years later (Larson et al., 2009). A cross-sectional study in Korean children demonstrated a strong positive association between the urinary concentrations of some phthalate metabolites and ADHD symptoms among school-age

children (Kim et al., 2009). This is the first study to focus on social behavior in school-aged children in relation to prenatal exposure to BPA and phthalates.

The prenatal period is uniquely vulnerable to the effects of EDs on maternal and fetal sex hormones in brain development (Zoeller 2007; Gore and Crews 2009). Both estrogen and testosterone are major modulators of brain biochemistry and behavior. They regulate and interact with neurotransmitters and influence the structural and functional organization of the brain (Rubinow 1996). They also influence the differentiation of sexually dimorphic brain regions involved in behavior, learning, memory, mood and socialization (Breedlove 1994). Some studies have suggested that the child's gender modifies the effect of prenatal exposure to BPA and phthalates. Prenatal exposure to some phthalates was found to be associated with less male-typical behavior (i.e., toy and activity preferences, play styles) in 3 to 6 year old boys (Swan et al., 2009). Higher prenatal BPA urinary concentrations were associated with externalizing behaviors in female offspring at 2 years of age (Braun et al., 2009). We found no substantial interaction between sex and phthalate or BPA exposures. Our analysis was conducted using samples collected in the third trimester whereas the findings reported by Braun et al. and Swan et al. were in relation to urine metabolites measured at mid-pregnancy. We also believe the prenatal hormonal milieu affects broader aspects of cognition and memory that may overshadow sex differences (MacLusky and Naftolin, 1981; McCarthy, 2009).

Phthalates and BPA appear to interfere with thyroid hormone regulation, which may also explain the lack of sex-specific associations between prenatal exposure and childhood behavior (Crofton et al. 2008; Ghisari et al. 2009; Zoeller et al. 2005). Meeker et al. (2007) reported an inverse association between phthalates and thyroxine (T4) levels in adult men (Meeker et al., 2007). Low serum free T4 in a cohort of pregnant women was associated with high urinary concentration of monobutyl phthalate, a metabolite of dibutyl phthalate during the second trimester (Huang et al., 2007). BPA appears to bind to the thyroid receptor (TR) and significantly inhibits TR-mediated gene activation at doses as low as 10 μM (Moriyama et al. 2002); BPA acted as a selective thyroid receptor antagonist in both rat pups (Zoeller et al. 2005) and in mouse oligodendrocyte precursor cells (Seiwa et al. 2004). Nakamura et al. (2006) found that prenatal exposure to low-doses of BPA in pregnant mice altered thyroid receptor expression in the fetal neocortex. The consequences of subclinical perinatal hypothyroxinemia on neurological outcomes in the offspring are well described (Haddow and Thyroid Study Group 2005; Pop and Vulsma 2005; Berbel et al. 2009). Experimental animal models have shown that transient intrauterine deficits of thyroid hormones result in permanent alterations of cerebral cortical architecture consistent with those observed in brains of patients with autism (Roman 2007; Sadamatsu et al., 2006).

Many of the families recruited into the original cohort were difficult to locate at the 7 to 9 year visit due to changes in residence or contact information. Despite the attrition that occurred over the ten year study period, we do not believe selection bias would account for our findings. There were no significant differences with respect to median urine concentrations of phthalate metabolites although BPA urine concentrations were slightly lower in the follow up cohort. One of the inherent limitations of using a parent rating survey is the potential for parental reporting bias. Although parents may over- or underreport symptoms on the SRS, misclassification on the SRS alone is not likely to result in bias of the effect estimates unless it is also differential by exposure (i.e. highly exposed more likely to over-report). This may be true, but would be predicated on the assumption that women knew their exposure levels. Mothers in our study were not aware of their phthalate metabolite or BPA urine concentrations; moreover, the study staff conducting interviews was also unaware of maternal exposure levels. An alternative explanation could be that our study population was in some way uniquely susceptible to the effects of endocrine disruption, i.e.,

a population at higher risk for childhood neurobehavioral or neuropsychiatric disorders. If mothers who noted problems with their children were more likely to return for clinic assessments (perhaps in order to receive free and independent evaluations of their child) and these children were more uniquely susceptible to the effects of EDs, then our effect estimates may be over-estimated (i.e. exposure-outcome effect of those who returned is different than that of the study base). This rationale is at best speculative, however, given that very little is known about susceptibility factors underlying ED exposures and effects. It is, therefore, essential that these results are replicated in an independent population, preferably one at lower risk for neurobehavioral disorders in childhood.

Both BPA and phthalates have short biological half-lives, therefore a single urine sample in third trimester may not adequately reflect long-term exposure levels to these chemicals and only represents one time point along a continuum of brain development; however, in the case of phthalates, urine biomarker measurements appear to be stable over periods of days to months perhaps due to consistent patterns of exposure to phthalate-containing personal care products (Adibi et al., 2008). Furthermore, the proliferation, migration and differentiation of certain cell populations within the cerebellum and hippocampus and ongoing developmental processes such as myelination and synaptogenesis are particularly sensitive to thyroid hormone status during the latter half of pregnancy (Howdeshell 2002; Bernal 2005). The neurodevelopmental endpoints we observed may also characterize exposures occurring during the major brain growth spurt that begins in the third trimester (Dobbing 1974).

Our study did not rely on the clinical diagnosis of ASD but only on symptoms common to the disorder. We can only report that phthalate metabolites were associated with autistic symptomatology in a non-referred cohort of healthy adolescents. With respect to MEP, for which we observed our strongest associations, there are currently four human studies suggesting its reproductive toxicity (Colon et al., 2000; Duty et al., 2003; Main et al., 2006; Swan et al., 2005). The parent diester of MEP, diethyl phthalate, is widely used in personal care products with fragrances and therefore MEP may serve as a marker of overall phthalate exposure or as a surrogate for other potentially toxic ingredients present in the same products.

Linkage studies and monozygotic twin concordance rates for autism attest to a predominantly genetic mode of inheritance; however, broader phenotypes that include communication and social disorders suggest that epigenetic and environmental factors may contribute to the variable expression of autistic-like traits (Muhle et al. 2004). Even when the level of social impairment falls below the threshold for an ASD, any existing impairment can intensify behavior problems and psychiatric conditions other than autism and PDD-NOS (Constantino, Hudziak, & Todd 2003). Social impairment is considered a reliable predictor of long-term outcome in both ASD and ADHD and without targeted interventions these social deficits often increase with age (Soorya and Halpern 2009). While the effect sizes we observed were relatively modest, even small perturbations in neurocognitive development at the individual level translate into clinically evident impairment across the population (Bellinger 2007).

Our findings also suggest an association between LMW phthalates and more autistic-like behavior on the subscales of Social Communication, Social Cognition, and Social Awareness. These subscales represent facets of the same underlying deficit in reciprocal social behavior. The milder behavior problems encompassed by these subdomains may be more relevant to an otherwise healthy population such as our study sample, while classic autistic behaviors captured by the Social Motivation and Autistic Mannerisms subscales may be more characteristic of a referred population (Constantino and Gruber 2005).

BPA-containing baby bottles are being phased out in many parts of the country and the 2008 Consumer Product Safety Improvement Act banned several phthalates from children's toys; however, BPA and phthalates are still widely used in many consumer goods and are rarely listed on labels of product ingredients (CERHR 2007; NRC 2008). Furthermore, mixtures of phthalate compounds and repeated daily exposures to both phthalates and BPA may potentiate their individual effects (Gray et al. 2006; NRC 2008). Finally, ongoing exposure to these endocrine disruptors in the postnatal period may be independently associated or may act cumulatively with prenatal exposure to increase the risk of atypical childhood behavior. Regulatory policies must account for the aggregate of mechanisms, pathways and mixtures that may give rise to these subtle health effects.

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Abbreviations

ASD	Autism Spectrum Disorder
BBP	Benzylbutyl Phthalate
BPA	Bisphenol A
CI	Confidence Interval
DEHP	Di(2-ethylhexyl) Phthalate
DEP	Diethyl phthalate
DBP	Dibutyl phthalate
ED	Endocrine disruptor
HMW	High molecular weight/ Phthalate
LMW	Low molecular weight/ Phthalate
MBP	Monobutyl Phthalate
MiBP	Mono-iso-butyl Phthalate
MBzP	Monobenzyl Phthalate
MCPP	Mono(3-carboxypropyl) Phthalate
MEP	Monoethyl Phthalate
MECPP	Mono(2-ethyl-5-carboxypentyl) Phthalate
MEHP	Mono(2-ethylhexyl) Phthalate
MEHHP	Mono(2-ethyl-5-hydroxylhexyl)-Phthalate
MEOHP	Mono(2-ethyl-5-oxohexyl) Phthalate
MMP	Monomethyl Phthalate
NHANES	National Health and Nutrition Examination Survey
PDD-NOS	Pervasive Developmental Disorder-Not Otherwise Specified
SD	Standard Deviation
SRS	Social Responsiveness Scale

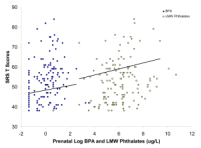

Figure 1. Prenatal BPA and LMW Phthalate urine biomarkers in relation to SRS T Scores at age 7 to 9 years

Scatter plot of total SRS scores and log urinary LMW phthalate and BPA levels (both in μg/L) for each study participant; the regression lines are adjusted for child race, sex, caretaker marital status and urinary creatinine as described in Table 4. The intercept represents the total SRS score among white women who are married or living with partner, and the index child is female. The urinary creatinine and toxicant levels are centered at the geometric mean.

Table 1

Comparison of Maternal Characteristics in Original Birth Cohort (N = 404) with those at 7 to 9 Year Follow Up (N = 137)

	Original Cohort		Follow Up Cohort	
Characteristics of Population	**N**	**%**	**N**	**%**
Maternal Age at Enrollment (years)				
< 20	142	35.1	47	34.3
20 – 24	132	32.7	50	36.5
25 – 29	44	10.9	16	11.7
≥ 30	86	21.3	24	17.5
Maternal Race/Ethnicity				
White	86	21.3	21	15.3
Black	112	27.7	45	32.9
Hispanic	200	49.5	71	51.8
Other	6	1.5	0	0
Maternal Education				
< High School	118	29.2	18	13.1
High School	83	20.5	26	19.0
Some College	103	25.5	58	42.3
≥ College Degree	100	24.8	30	21.9
Marital Status at Enrollment				
Married	117	29.0	36	26.3
Living w/ Baby's Father	98	24.3	23	16.8
Single/Divorced/Widowed	189	46.8	76	55.5
Smoke during Pregnancy (ever)	67	16.6	8	5.2
Alcohol during Pregnancy (ever)	59	14.9	23	15.0
Mother is Primary Caretaker			112	73.2
Breastfeeding				
< 1 month			61	43.9
1-4 months			43	30.9
>4 months			35	25.2

Table 2

Comparison of Median Maternal Biomarker Concentrations in Original Birth Cohort with those at 7 to 9 Year Follow Up.

Urine Biomarker	N	Original Cohort, μg/L (IQR)	N	Follow Up Cohort, μg/L (IQR)
BPA	404	1.3 (0.7–2.3)	134	1.2 (0.5–2.0)
ΣHMW Phthalates[a]	404	120 (61–250)	137	125 (49–278)
ΣLMW Phthalates[b]	404	430 (175–1,090)	137	419 (158–1,015)
MEP		380 (137-1,010)		372 (130–964)
MBP		36 (16-75)		33 (15–87)
MiBP		6.2 (2.7-12)		6.5 (2.9–15)
MMP		1.6 (1-3.8)		1.8 (0.7–3.9)

IQR = Interquartile Range;

[a] ΣHMW Phthalates = DEHP metabolites [MECPP, mono(2-ethyl-5-carboxypentyl) phthalate; MEHHP, mono(2-ethyl-5-hydroxylhexyl) phthalate; MEOHP, mono(2-ethyl-5-oxohexyl) phthalate and MEHP, mono(2-ethylhexyl) phthalate], MBzP, monobenzyl phthalate and MCPP, mono(3-carboxypropyl) phthalate.

[b] ΣLMW phthalates = MMP, monomethyl phthalate; MEP, monoethyl phthalate; MBP, monobutyl phthalate; and MiBP, mono-isobutyl phthalate).

Table 3

Prenatal Biomarker Concentrations (μg/L) in Relation to Severity of Social Impairment[a]

Clinical Category (SRS *T*-Score)	N	LMW Phthalates Median, μg/L(IQR)	HMW Phthalates Median, μg/L(IQR)	N	BPA Median, μg/L(IQR)
Normal Range (< 60)	106	328 (147 - 831)	170 (65 - 362)	103	1.10 (0.50 - 2.10)
Mild Social Impairment (60–74)	25	460 (224 - 1124)	159 (92 - 316)	25	1.40 (0.90 - 2.00)
Severe Social Impairment (≥ 75)	6	1260 (544 - 2863)	318 (160 - 433)	6	1.45 (1.00 - 2.00)

IQR = Interquartile Range

[a]Wilcoxon rank-sum tests comparing the medians of mild + severe social impairment versus normal range were not significant. (LMW phthalates, p = 0.09; HMW phthalates, p = 0.54; BPA, p = 0.24).

Table 4

Beta Coefficients and 95% Confidence Intervals for Prenatal Urine Biomarkers in Relation to Social Responsiveness Scale Scores (N = 137).

SRS *T*-Scores	ΣLMWP[a]	MBP	MEP	MMP	ΣHMWP[b]	ΣDEHP[c]	BPA (N = 134)	BPA (N = 128)[d]
Total Score	1.53 (0.25, 2.82)*	1.37 (-0.43, 3.17)	1.38 (0.23, 2.53)*	1.29 (-0.65, 3.24)	0.84 (-0.81, 2.48)	0.83 (-0.69, 2.35)	1.18 (-0.75, 3.11)	1.73 (0.02, 3.45)*
Subscale Scores								
Cognition	1.40 (0.07, 2.74)*	1.24 (-0.62, 3.10)	1.28 (0.10, 2.47)*	1.69 (-0.31, 3.68)	0.64 (-1.06, 2.34)	0.76 (-0.81, 2.33)	0.82 (-1.16, 2.81)	1.50 (-0.22, 3.22)
Communication	1.86 (0.48, 3.24)*	1.85 (-0.08, 3.78)	1.67 (0.44, 2.90)*	1.32 (-0.77, 3.42)	0.87 (-0.89, 2.64)	0.83 (-0.81, 2.47)	1.00 (-1.09, 3.09)	1.77 (-0.01, 3.56)
Mannerisms	0.88 (-0.50, 2.26)	1.3 (-0.60, 3.21)	0.77 (-0.46, 2.00)	1.59 (-0.46, 3.64)	0.73 (-1.01, 2.47)	0.46 (-1.16, 2.07)	1.17 (-0.88, 3.22)	1.39 (-0.52, 3.30)
Motivation	0.83 (-0.35, 2.02)	0.28 (-1.36, 1.92)	0.77 (-0.28, 1.83)	0.25 (-1.52, 2.03)	0.59 (-0.90, 2.08)	0.56 (-0.82, 1.94)	0.82 (-0.93, 2.57)	0.95 (-0.79, 2.68)
Awareness	1.25 (0.09, 2.42)*	0.63 (-1.01, 2.26)	1.10 (0.06, 2.14)*	0.19 (-1.58, 1.95)	0.66 (-0.82, 2.15)	0.90 (-0.47, 2.28)	1.35 (-0.41, 3.10)	1.62 (-0.17, 3.40)

Adjusted for child race, sex, caretaker marital status and urinary creatinine.

[a] ZLMW phthalates = MMP, MEP, MBP and MiBP.

[b] ΣHMW = MBzP, MCPP and

[c] ΣDEHP [MECPP, MEHHP, MEOHP and MEHP].

[d] excluded outliers.

* $p < 0.05$