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Prenatal Exposure to Bisphenol A and Phthalates and Infant Neurobehavior

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Abstract

Objective—To examine the association of prenatal exposure to bisphenol A and select common phthalates with infant neurobehavior measured at 5 weeks.

Methods—We compared the concentration of maternal urinary metabolites of bisphenol A and phthalates at two distinct time points in pregnancy (16w, 26w) with scores on the NICU Network Neurobehavioral Scale (NNNS) at 5 weeks of age in a cohort of 350 mother/infant pairs.

Results—Prenatal exposure to BPA was not significantly associated with neurobehavioral outcomes at 5 weeks. Significant associations between prenatal exposure to measured phthalates and infant neurobehavioral outcomes differed by type of phthalate and were only seen with exposure measured at 26 weeks. Higher total di-butyl phthalate (DBP) metabolites at 26w was associated with improved behavioral organization evidenced by decreased arousal (p=.04), increased self-regulation (p=.052), and decreased handling (p=.02). In males, higher total di-2-ethylhexyl phthalate (DEHP) metabolites at 26w was associated with more nonoptimal reflexes (p=.02).

Conclusion—The association between prenatal phthalate exposure and infant neurobehavior differed by type of phthalate and was evident only with exposure measured at 26w. Prenatal exposure to DBP was associated with improved behavioral organization in 5-week-old infants. Prenatal exposure to DEHP was associated with nonoptimal reflexes in male infants. There was no evidence of an association between prenatal BPA exposure and infant neurobehavior.

Keywords

Bisphenol A; Infant neurobehavior; Phthalates; Prenatal exposure

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

1. Introduction

Synthetic materials are produced in great volume in industrialized countries, and they are a part of our daily lives in the United States. Many of these chemicals have been called into question regarding their safety and potential harmful effects on health, development, and behaviors, especially among infants and children who are often more vulnerable to exposure than adults (Bearer, 2000; Vorhees, 1986). Bisphenol A (BPA) and phthalates are found in many manufactured products with nearly universal human exposure (Centers for Disease Control and Prevention, 2009). Both BPA and phthalates are becoming increasingly scrutinized by the research community and the public yet limited conclusive evidence has been gathered to support concerns or endorse the safety of these chemicals.

1.1 Bisphenol A

Bisphenol A (BPA) is a high-production synthetic chemical used in the manufacture of polycarbonate plastics and epoxy resins. These materials can be found in reusable water bottles, food cans and containers, compact discs, flooring, and paints. Recent reports also indicate the potential for transferability of BPA from cash register receipts (Biedermann, et al., 2010). National data from 2003-2004 indicated that 93% of the US population over age five years had measureable BPA metabolites in urine (Calafat, et al., 2008). The primary mode of exposure for adults is through ingestion of foods in contact with BPA containing materials. Pregnant women who regularly consume canned vegetables, work as cashiers, or are exposed to tobacco smoke have higher urinary BPA concentrations than pregnant women without these characteristics (Braun, et al., 2010). Bottle-fed infants and children are more likely to be exposed to BPA through polycarbonate baby bottles (von Goetz, et al., 2010) although these are no longer manufactured in the US. Median urinary concentrations of total BPA were approximately two-fold higher among infants fed from bottles than other infants (Völkel, et al., 2010). BPA has weak estrogenic properties (Akingbemi, et al., 2004; Lee, et al., 2003) and appears to interfere with typical development of male and female brains, affecting behavior patterns related to reproduction and social and non-social behaviors (Palanza, et al., 2008). Animal studies have demonstrated detrimental effects of BPA on reproductive function (Salian, et al., 2009), on maternal behavior (Palanza, et al., 2002), on increased body weight in females (Adewale, et al., 2010; Somm, et al., 2009), and on sex-typical behaviors (Palanza, et al., 2008). Human studies have linked environmental BPA exposure with reduced testosterone (Mendiola, et al., 2010) and infertility (Li, et al., 2010; Meeker, et al., 2010) in adult men, as well as fewer oocytes and serum estradiol levels in women undergoing in-vitro fertilization (Mok Lin, et al., 2010).

Animal studies also indicate an association between prenatal and early postnatal BPA exposure and neurobehavioral effects such as increased anxiety (Poimenova, et al., 2010; Tian, et al., 2010) and cognitive deficits (Poimenova, et al., 2010; Tian, et al., 2010; Xu, et al., 2010) that persist into adulthood and appear to be permanent (Xu, et al., 2010). Palanza (Palanza, et al., 2008) demonstrated that behaviors that are typically sexually dimorphic were no longer different between male and female mice as females exposed to BPA behaved more like control males. We have previously reported an association between prenatal exposure to BPA and increased externalizing behaviors in two-year-old girls (Braun, et al., 2009). Miodovnik (Miodovnik, et al., 2010) recently reported a lack of association between prenatal BPA exposure and social impairment among school age children. We know of no published studies of BPA and early infant neurobehavioral outcomes.

1.2 Phthalates

Phthalates are a family of synthetic compounds used in the production of some plastics to enhance flexibility and resilience. They can also be found in personal care products such as

cosmetics, perfumes, and shampoos as well as in polyvinyl chloride household and industrial products such as shower curtains, upholstery, toys, and medical equipment. Because they are present in a wide variety of industrial, household, and personal care products, human exposure is nearly universal (Centers for Disease Control and Prevention, 2009). Phthalates enter the body through inhalation, ingestion, and dermal contact. They are rapidly hydrolyzed into monoesters, metabolized further, and excreted in urine and feces (Wormuth, et al., 2006).

There is evidence that some phthalates may have anti-androgenic activity. Animal research strongly implicates high-dose exposure to some phthalates in altered reproductive function in rats such as decreased anogenital distance (Gray Jr, et al., 2000; McKee, et al., 2006; Moore, et al., 2001; Parks, et al., 2000) and undescended testes in males (Moore, et al., 2001), reduced testosterone production (Parks, et al., 2000) and sperm production (Andrade, et al., 2006; Dalsenter, et al., 2006; McKee, et al., 2006) in males, delayed puberty in males (Noriega, et al., 2009) but earlier puberty in females (Ma, et al., 2006), and sexual inactivity in males (Lee, et al., 2006; Moore, et al., 2001). In humans, similar reproductive outcomes have been reported including decreased anogenital distance in boys (Lottrup, et al., 2006; Swan, et al., 2010), and in adult males, there is evidence of altered semen quantity and quality (Hauser, et al., 2006; Pant, et al., 2008), DNA damage to sperm (Hauser, et al., 2007), and changes to hormone levels (Meeker, et al., 2007; Meeker, et al., 2009). Weak and conflicting relationships have been reported regarding pubic hair and breast growth in girls including a positive association with some phthalates and a negative association with other phthalates (Wolff, et al., 2010).

There is modest but growing evidence linking exposure to some phthalates with neurobehavioral outcomes. In animals, reported neurobehavioral deficits include impaired righting ability (Li, et al., 2009; Tanaka, 2002), weakened grasp strength and grip time (Li, et al., 2009), and impaired spatial learning and reference memory revealed by delays in completing mazes (Li, et al., 2009; Tanaka, 2005). Additional behavioral associations include increased hyperactivity (Ishido, et al., 2004) and decreased grooming behavior (Hoshi, et al., 2009). Many of these neurobehavioral deficits also have greater impact on male than female animals.

Published studies of neurobehavioral outcomes in humans following phthalate exposure are extremely limited. Reported outcomes include reduced masculine play in preschool boys (Swan, et al., 2010), more parent-reported behavior problems such as aggression, conduct disorder, attention problems, depression, less emotional control and executive function skills at four to nine years (Engel, et al., 2010), and deficits in social functions, (Miodovnik, et al., 2010) reduced intelligence (Cho, et al., 2010), and more frequent attention deficit hyperactivity disorder at school age (Kim, et al., 2009). In the only investigation of newborn neurobehavior, Engel reported associations between prenatal phthalate exposure, assessed by the urinary concentrations of phthalate metabolites measured once between 26 and 40 weeks gestation, and newborn neurobehavioral domains measured within the first five days with the Neonatal Behavioral Assessment Scale (NBAS) (Brazelton, et al., 1995). Significant associations varied by sex including decreased orientation and alertness in females and improved motor performance in males.

Given the extremely limited research on human neurobehavioral outcomes in association with prenatal exposure to chemicals present in plastics, and the potential for substantial public health impact due to the pervasiveness of plastics in our environment, our objective was to examine the association of prenatal BPA and phthalate exposure, measured at two distinct time points in pregnancy, with early infant neurobehavior measured at five weeks of

age using the NICU Network Neurobehavioral Scale (NNNS) (Lester, et al., 2004), a tool with proven sensitivity to both overt and subtle differences in infant neurobehavior.

2. Methods

2.1. Subjects

We included mother and infant pairs who were enrolled in the Health Outcomes and Measures of the Environment (HOME) Study. HOME Study enrollment criteria and procedures are more completely described elsewhere (Geraghty, et al., 2008). Briefly, from March 2003 to February 2006, 468 healthy pregnant women, at least 18 years of age, were enrolled in the study at 16 ± 3 weeks (mean ± standard deviation) gestation. Women resided in southwestern Ohio, received prenatal care from one of nine participating obstetrical clinics, and planned to deliver at one of three participating hospitals. Of the 468 enrolled women, 389 remained in the study and delivered live singletons, and we obtained NNNS examinations at five weeks on 355 (91.3%) of these infants. We further restricted the sample to women who had 16 week and 26 week urine samples collected within four weeks of the target collection point (e.g., 16w±4w, 26w±4w) to avoid outliers that could obscure estimates of exposure effects by time as well as overlap in the 16w and 26w urine collection window. This resulted in a final sample size of 350 mother/infant pairs. Institutional review boards of all involved research institutions, hospitals, and laboratories approved the study protocol.

2.2. Survey Data

Mothers were surveyed during pregnancy and at the five week visit for collection of demographic and socioeconomic status variables, reported smoking and exposure to second hand smoke, and use of illicit drugs and alcohol during pregnancy. Mothers also completed the Beck Depression Inventory (BDI-II) (Beck, et al., 1996) both prenatally and at five weeks to measure depression symptoms. Mothers scoring >13 on the BDI-II were coded as experiencing any depression in accordance with the BDI-II manual and were categorized as being ever or never depressed based on the two BDI administrations.

2.3. Medical Chart Review

We conducted a thorough review of both mother and infant medical records prior to discharge from the delivery hospital. We collected information about pregnancy course, labor and delivery, and newborn characteristics.

2.4. Urine Measures

Urine was collected from women at 16 ± 4 weeks (mean=16.0, SD=1.9) and 26 ± 4weeks (mean=26.5, SD=2.0) gestation to reflect exposures during distinct periods of pregnancy. Successful collection of samples varied by each time point as follows: 16w n = 346, 26w n = 332; 22 women had measurements at only one time point, 328 women had measurements at both times.

The urinary concentrations of BPA and phthalate metabolites were measured at the Centers for Disease Control and Prevention (CDC) Environmental Health Laboratories using published methods (Silva, et al., 2008; Ye, et al., 2005). The limit of detection (LOD) for BPA was 0.4 ng/mL, and both BPA and phthalate measures include concentration of total (free plus conjugate) species. The LODs for the six phthalate metabolites of interest ranged from 0.3 to 1.2 ng/mL. We grouped the six measured phthalate metabolites as follows: di-butyl phthalate (DBP) = mono-n-butyl phthalate (MnBP), and mono-isobutyl phthalate (MiBP); di-2-ethyhexyl phthalate (DEHP) = mono-2-ethyl-5-carboxypentyl phthalate (MECPP), mono-2-ethyl-5-carboxyhexyl phthalate (MEHHP), mono-2-ethyl-5-oxohexyl

phthalate (MEOHP), and mono-2-ethylhexyl phthalate (MEHP). Specifically, the DBP and DEHP summary measures were calculated as totals of the measured metabolites in molar concentration (nmol/L). Imputed values using LOD/square root of 2 (Hornung, 1991) were used for any metabolite levels below the LOD. Both BPA and the phthalate metabolite measures were transformed using $\log_2$ to normalize their distributions for analysis.

2.5. Early Infant Neurobehavior

Early infant neurobehavior was measured using the NNNS (Lester and Tronick, 2004) during a home visit at approximately five weeks after delivery. The NNNS is a comprehensive neurobehavioral assessment that evaluates neurological functioning, provides a behavioral profile, and measures signs of stress in young infants. It is appropriate for infants 30 to 46 weeks gestational age but has most commonly been used around four weeks after birth. The instrument is especially sensitive to the capabilities and vulnerabilities of at-risk infants such as those born prematurely or prenatally exposed to potentially neurotoxic substances. The typical NNNS exam requires about 30 minutes. It begins with an observation of baseline tone and respiration. A package of habituation items requiring the infant to be asleep is often omitted, especially in older newborns. The assessment includes evaluation of primitive reflexes, active and passive tone, movement quality, alertness and orientation to a variety of stimuli, and signs of stress/abstinence in response to the exam. Exams were completed by one of four certified examiners who were masked to all prenatal exposure information, including BPA and phthalate exposures. For 89% of the infants, the NNNS assessment was conducted in a quiet room apart from the mother who was simultaneously being interviewed by another staff member. For the remaining 11% of infants, the mother was in the same room during the NNNS assessment. Examiners are trained to focus only on the infant and tune out any possible distractions during the entire exam.

Analysis of raw NNNS data yields summary scores describing 13 dimensions of neurobehavior: habituation, attention, arousal, self-regulation, special handling required to acquire orientation items, movement quality, excitability, lethargy, non-optimal reflexes, asymmetrical reflexes, hypertonicity, hypotonicity, and stress/abstinence. For each subscale, higher scores indicate more of that quality during the exam regardless of whether it is a good or bad quality. For example, higher scores on self-regulation indicate that the infant was adept in regulating his own responses during the exam, a positive outcome, while higher scores on special handling indicate that the infant required more assistance from the examiner in order to complete the assessment, a poor outcome.

2.6. Statistical Analysis

Among the 350 infants with a completed five-week NNNS assessment and at least one prenatal urinary measure from their mothers for BPA and phthalate metabolites within the defined gestational periods, we initially examined the distribution of each variable using summary measures (mean, median, percentage, frequency). For continuous exposure variables, we used $\log_2$ transformation to normalize the data. We then conducted analysis of bivariate associations between urine BPA and total DBP and DEHP metabolite measures at the two separate collection times and NNNS outcomes, controlling for $\log_2$ creatinine. The hypotonicity scale was dichotomized and analyzed with logistic regression due to a distribution in which the majority of infants acquired a score of zero and few had values greater than one. The asymmetries scale was analyzed with Poisson regression. All other scales were analyzed with linear regression. For the NNNS scales that demonstrated significant associations with urinary concentrations of BPA and phthalate metabolites ($p<.10$), we then constructed multivariable models, using linear (arousal, regulation, handling, movement quality, nonoptimal reflexes scales) and logistic (hypotonicity scale) regression

analyses, for the associations at the two measurement times and each individual NNNS scale. In these multivariable models, associations were considered significant when $p < .05$. In all multivariable models, we retained $\log_2$ creatinine to account for urine concentration (Barr, et al., 2005), infant age in days at the time of exam, and sex as standard covariates. We also explored the following other potential covariates: maternal race, household income, marital status, maternal depression, maternal body mass index (BMI) at 13-19 weeks gestation, maternal blood lead level during pregnancy, reported alcohol use during pregnancy, reported marijuana use during pregnancy, maternal serum cotinine (a measure of tobacco smoke exposure) during pregnancy, infant weight change per month from birth to five weeks. In addition, we included a covariate that identified infants who could potentially be at high-risk for neurobehavioral deficits based on gestational age less than 37 weeks, birth weight less than 2500g, and/or stay in the neonatal intensive care unit (NICU) following birth. Covariates were retained in final models if they were significantly associated with neurobehavior ($p<.05$) or if removal changed the BPA/phthalate metabolite estimate by greater than 10%. Removal of covariates was done for each NNNS scale individually, with systematic removal of those covariates having the largest p-value first. Based on published findings of difference in effects of BPA and phthalates by sex in both animals and humans, we tested for sex interactions. As there is evidence of potential thresholds with amplified effects at low levels of exposure to environmental toxicants (Lanphear, et al., 2005; Yolton, et al., 2005), we examined the linearity of the relationship between the outcomes (NNNS scales) and the transformed exposure variables using scatter plots and residual plots. We found no evidence of non-linearity and thus did not conduct additional threshold analyses using cut-points of exposure.

3. Results

3.1. Description of the Sample

Of the 389 live singletons that were born into the study, 350 completed a five-week NNNS assessment and had at least one prenatal urine sample from their mothers analyzed for BPA or phthalate metabolites. Characteristics of the sample are shown in Table 1. Women averaged 30 years old at delivery of the infant. The majority were married, had greater than high school education, and were employed during pregnancy. Racial makeup of the women was 63% white, 30% black, and 7% other. Based on medical chart review, the mean gestational age of infants at delivery was 39 weeks, and mean birth weight was 3389 grams. Forty six percent of infants were male, and they averaged 34.5 days at the time of the five-week NNNS assessment.

3.2. BPA and Phthalate Measures

At least 90% of maternal urine samples had detectable concentrations of BPA at the two different measurement time points. All samples had detectable concentrations of phthalate metabolites for DBP and DEHP. (Table 2) BPA levels were slightly lower than national data on females reported by the CDC(Centers for Disease Control and Prevention, 2009), but were strikingly similar to those reported in other studies (Cantonwine, et al., 2010; Wolff, et al., 2008; Ye, et al., 2008). Most of the phthalate metabolite levels were substantially higher than those of national samples (Centers for Disease Control and Prevention, 2009). The Pearson correlation of $\log_2$ urine BPA concentrations across the two collections times was $r = 0.29$ ($p <0.0001$). The Pearson correlation of $\log_2$ urine phthalate metabolite concentrations across the two collection times were $r = 0.41$ ($p <0.0001$) for DBP, and $r = 0.24$ ($p <0.0001$) for DEHP. The Pearson correlations of $\log_2$ BPA with $\log_2$ phthalate metabolites (DBP, DEHP) at 16 weeks were $r = 0.50$ and $r = 0.42$, respectively, and at 26 weeks were $r = 0.28$ and $r = 0.21$, respectively (all $p < 0.0001$).

3.3. Early Infant Neurobehavior

The number of infants receiving scores on the habituation and hypertonicity scales of the NNNS was too small for meaningful interpretation, so we excluded these scales from analyses as we have in our previous work with the NNNS (Yolton, et al., 2009). Mean scores of the NNNS subscales for the sample were within the ranges reported by Tronick (Tronick, et al., 2004) and Lester (Lester, et al., 2004). Table 3 displays bivariate associations between our exposure measures and NNNS subscales. The only significant ($p < 0.10$) bivariate association between BPA concentration in urine and NNNS outcomes, controlling for urinary creatinine, was with the 16 week sample and increased hypotonia. To further describe associations with BPA exposure, we conducted bivariate analyses of urinary BPA concentrations and descriptive characteristics among the sample. Maternal urinary BPA concentrations were significantly associated with several covariates. Higher BPA concentrations were associated with higher levels of maternal urine creatinine, serum cotinine, BMI, and with black race. Maternal BPA concentrations were inversely associated with income and single marital status.

In initial analyses with total urinary DBP metabolite concentrations where we only controlled for urinary creatinine (Table 3), associations significant at $p < .10$ included: increased hypotonia with higher total DBP metabolite concentrations at 16 weeks; decreased arousal, higher regulation, decreased handling, higher movement quality, and increased hypotonia scores with higher total DBP at 26 weeks. In similar analyses with the total DEHP metabolite concentrations controlling for urine creatinine, we found a significant positive association at 26 weeks with more nonoptimal reflexes.

3.4. Multivariable Analyses

We conducted multivariate analyses of the associations between total maternal urinary concentrations of BPA, DBP, and DEHP metabolites at the two independent measurement times and NNNS outcomes for those significant at $p < .10$ in the preliminary analyses where we had only controlled for urinary creatinine. Final multivariable model associations are presented in Table 4.

In multivariable analysis, the association between 16 week BPA and hypotonia reflected a trend toward increased hypotonia, or decreased muscle tone, that was not significant ($p = .09$). This was the only association with BPA exposure that we explored with multivariable analysis.

In multivariable analyses, the associations between total DBP metabolites at both 16 and 26 weeks and hypotonia were not significant [OR=1.20 (95% CI: 0.96 – 1.50) and OR=1.22 (95% CI: 0.96 – 1.55), respectively]. When measured at 26 weeks, higher urinary concentrations of DBP metabolites were significantly associated with lower arousal, higher self-regulation (trend), less handling required, and improved movement quality (trend) during the NNNS. We found a significant interaction of sex by urinary DEHP metabolites measured at 26 weeks with respect to non-optimal reflexes ($p = .04$). A sex-stratified analysis revealed a significant association between DEHP urinary metabolites and increased nonoptimal reflexes in males only. Log_2 creatinine, age at exam, and infant sex were retained in all final models regardless of their impact. Models were relatively parsimonious with other retained covariates including weight change from birth (arousal, regulation, nonoptimal reflexes), maternal alcohol use (arousal, regulation), maternal maximum log_2 cotinine (arousal), marital status (regulation, movement quality), high risk infant status (regulation, movement quality), income (nonoptimal reflexes), and black race (hypotonia).

4. Discussion

In this study of prenatal exposure to selected plastic materials and subsequent neurobehavior measured at five weeks of age, we found no significant associations with BPA exposure during gestation. Significant associations with DBP and DEHP exposure only occurred with exposure measured at 26 weeks and suggested both favorable and detrimental outcomes, but results differed by type of phthalate. Increased levels of DBP metabolites at 26 weeks were significantly associated with decreased arousal and decreased handling required, and trends toward improved regulation and movement quality. However, increased DEHP metabolites were associated with increased non-optimal reflexes in boys only.

4.1 BPA and Early Infant Neurobehavior

While prenatal exposure to BPA has been associated with behavioral and cognitive effects in animals and humans (Braun, et al., 2009; Palanza, et al., 2008; Poimenova, et al., 2010; Tian, et al., 2010; Xu, et al., 2010), this is the first study to examine early infant behaviors in relation to BPA exposure. We found no significant associations between gestational exposure to BPA and infant neurobehavior measured at five weeks, but we did see a trend toward greater hypotonia with higher BPA exposure at 16 weeks gestation. Our findings are not consistent with previous studies of behavioral and cognitive associations with gestational exposure to BPA. This could be due to the low levels of exposure that were detected or to the nature of the outcome measured. The levels of BPA in our sample were slightly lower than those reported nationally for females but very similar to the levels reported for pregnant women in the United States, Mexico, and the Netherlands (Cantonwine, et al., 2010; Wolff, et al., 2008; Ye, et al., 2008). It is possible that our exposures were below a threshold at which neurobehavioral effects would be evident, but no such threshold has yet been recognized for BPA, and Braun (Braun, et al., 2009) observed BPA-associated behaviors in two-year-old children from this same cohort. The NNNS is a very early measure of inherent neurobehavioral traits in the young infant. The measures employed at later ages incorporate innate characteristics as well as experiences the child may have during the course of development. Additional longitudinal studies will be necessary to carefully assess the true threat posed by BPA and to examine the point at which any significant neurobehavioral impact becomes apparent. For the time being, we suggest these results be interpreted with caution.

4.2 Phthalates and Early Infant Neurobehavior

In this study, exposure to DBP and DEHP during pregnancy was significantly associated with several neurobehavioral outcomes as measured by the NNNS at five weeks of age. We found both favorable and detrimental associations with infant neurobehavior that differed by the type of phthalate. All of our significant findings were associated with exposures that occurred around 26w gestation. Higher urinary concentrations of DBP metabolites in pregnant women were generally associated with improved behavioral organization among their infants. More specifically, higher urinary concentrations of DBP were significantly associated with lower scores on NNNS scales describing infant arousal and special handling required during the exam. We also noted trends toward improved self-regulation and movement quality with greater urinary concentrations of DBP measured at 26 weeks. Higher DEHP metabolite levels in pregnant women were associated with more nonoptimal reflexes in male infants.

Higher urinary concentrations of DBP metabolites were associated with lower scores on the arousal scale. The arousal scale on the NNNS is fairly complex. It measures the infant's general level of arousal during the exam as well as specific aspects such as associated motor activity and irritability during the assessment. Infants who score high on the arousal scale

quickly become overstimulated and irritable, and they tend to fuss/cry and exhibit excessive motor movements through much of the exam. In general, a lower level of arousal is preferable and demonstrates more stability in behavior, but extremely low arousal can also be seen as problematic as the infant may become unresponsive to stimulation. The arousal scale appears to be quite sensitive to exposures and experiences during gestation. Lower arousal has been associated with prenatal exposure to methamphetamine (LaGasse, et al., 2010), cocaine (Lester, et al., 2002), tobacco (Yolton, et al., 2009), and maternal depression (Paz, et al., 2009) in other studies. Increased arousal has been associated with prenatal exposure to marijuana (de Moraes Barros, et al., 2006) and tobacco (Yolton, et al., 2009). It is unclear at what point on the NNNS arousal scale that lower scores become problematic and whether reduced arousal may actually be an adaptive response by the infant to avoid overstimulation.

Higher urinary concentrations of DBP metabolites were associated with lower scores on the handling scale. This scale pertains specifically to efforts used by the examiner to maintain infant focus and cooperation during the orientation package of the NNNS. Efforts can be made by the examiner to either increase alertness or to soothe the infant due to irritability. Lower scores on this scale indicate that the infant required minimal assistance from the examiner to perform optimally. Lower handling scores have been associated with maternal depression (Paz, et al., 2009) while higher handling scores have been associated with prenatal exposure to tobacco smoke (Law, et al., 2003; Stroud, et al., 2009).

Consistent with lower scores on arousal and handling scales, we also found higher DBP exposure was associated with a trend toward higher scores on the regulation scale of the NNNS. This scale combines physiological, motor, and attentional activation, and soothability. It generally measures the infant's ability to cope with the demands of the exam. Higher scores indicate better regulation. Poorer ability to self-regulate has been associated with prenatal exposure to marijuana (de Moraes Barros, et al., 2006), cocaine (Lester, et al., 2002), and tobacco (Yolton, et al., 2009).

Higher urinary concentrations of DBP metabolites were associated with a trend toward improved movement quality. Engel (Engel, et al., 2009) also reported an association between prenatal low-molecular-weight phthalate exposure, such as DBP, and improved motor performance but in males only. We did not find a significant sex by phthalate interaction with respect to DBP. Decreased movement quality has been reported in association with prenatal cocaine exposure (Lester, et al., 2002).

Higher urinary concentrations of DEHP metabolites were associated with a higher frequency of non-optimal reflexes in males. The NNNS includes a battery of reflexes in both upper and lower extremities, as well as upright, prone, and supine responses. Reflexes that are not within the standard optimal range are coded as non-optimal and can represent hypo- or hyper-reflexive responses. The NNNS manual suggests that the number of non-optimal reflexes is likely more important than performance on any single reflex item (Lester and Tronick, 2004). Reflexes during early infancy are linked with prenatal exposures to cocaine (Law, et al., 2003).

4.3 Timing of Exposure Assessment

In a study of methamphetamine use during pregnancy (LaGasse, et al., 2010), neurobehavioral outcomes in newborns assessed by the NNNS varied greatly between those who were exposed in the first trimester compared with the third trimester. Braun (Braun, et al., 2009) also reported that associations between maternal urinary concentrations of BPA and child behaviors at age two years varied by timing of exposure assessment during gestation. These studies highlight the importance of timing of exposure assessment. In the

current study, the 26 week assessment point was the most sensitive for phthalate exposure and neurobehavioral associations. We found significant associations between maternal DBP and DEHP metabolite concentrations at 26 weeks and lower arousal, improved regulation, decreased handling, improved movement quality, and more nonoptimal reflexes in male infants.

We did not find any significant associations with 16 week phthalate measures. We found one nonsignificant trend between maternal BPA concentrations at 16 weeks with increased hypotonia. In a previous study of BPA exposure, the 16-week exposure point was found to be most highly associated with female behavior problems at age 2 years (Braun, et al., 2009). However, with respect to phthalates, our findings suggest that exposure during the 26-week time window is more important when considering outcomes during early infancy. These findings should be interpreted with caution until they can be replicated.

4.4 Type of Phthalate Exposure

In this study, prenatal exposure to DBP, a compound for which an important exposure source includes personal care products like cosmetics, shampoos, and perfumes, was primarily associated with favorable outcomes at five weeks including decreased arousal and decreased special handling needed to complete the exam. In addition, there were trends toward improved self-regulation and movement quality with higher DBP exposure. The findings related to these NNNS scales suggest an association in which infants are more regulated and controlled during the assessment and require less assistance from the examiner in completing the assessment. Exposure to DEHP, which is primarily a plasticizer of polyvinyl chloride found in household furnishings, medical supplies, and packaging products, was associated with more non-optimal reflexes but in males only. All these associations were observed in relation to exposure measured at 26 weeks.

In the only other published analysis of prenatal phthalate exposure and infant neurobehavior, Engel (Engel, et al., 2009) also reported differential associations with neonatal neurobehavior by the type of phthalate. Exposure to low molecular weight phthalates, such as DBP, was associated with improved motor performance in boys while exposure to high molecular weight phthalates, such as DEHP, was associated with lower scores on orientation and alertness in girls. The findings from both studies suggest that exposure to high molecular weight phthalates may be associated with more potentially damaging neurobehavioral outcomes in young infants while exposure to low molecular weight phthalates may be associated with more positive neurobehavioral outcomes even though exposure to DBP has been associated with subtle anatomical changes in infant boys (Swan, et al., 2010).

The minimal studies of cosmetic use during pregnancy indicate measured DEP metabolites are significantly greater among those women who use personal care products such as perfumes, deodorants, lipstick, and lotions but make no links with pregnancy outcomes (Berman, et al., 2009; Just, et al., 2010). Research has demonstrated increased risk for spontaneous abortion among cosmetologists who are regularly exposed to phthalates and other chemical compounds (John, et al., 1994). Wolff (Wolff, et al., 2008) reported increased risk for lower birth weight but increased gestational age and head circumference, but others have reported higher risk of preterm birth (Meeker, et al., 2009; Whyatt, et al., 2009) among babies born to women with higher phthalate exposure during pregnancy. None of these studies identified specific patterns of use of personal care products and cosmetics during pregnancy. Additional research into patterns of cosmetic use during pregnancy and subsequent offspring neurobehavioral outcomes may help us better understand phthalate exposure and the conflicting outcomes seen in the current research.

4.5 Sex Differences

Animal studies and a small number of human studies suggest that phthalates may affect males and females differently. Engel (Engel, et al., 2009) reported deficits in attention and alertness in females with increased exposure to some high-molecular-weight phthalates such as DEHP, and improved motor performance in males with increased exposure to low-molecular-weight phthalates such as DBP. We found only one significant sex by phthalate interaction in our analyses. In sex-stratified analysis with urinary metabolites of DEHP measured at 26 weeks, we found a significant association with more nonoptimal reflexes among males only. While this finding does not coincide with those of Engel, the infants in Engel's study were assessed within the first 5 days after delivery and may represent an earlier impact of phthalate exposure. In addition, Engel reports measuring urinary concentrations of phthalate metabolites across a range of 25 to 40 weeks gestation while we have distinct measurement periods at 16±4 and 26±4 weeks. The narrow ranges of our sample collection windows may allow more specificity in identifying associations.

5. Limitations

The study has several limitations. The date of mother's last menstrual period was used to estimate gestational age and can be an inexact measurement tool in determining the point of gestation. We do not have both time-point measures from all women, but have them both for 94%. BPA and phthalate metabolites have short half-lives, and our exposure measurements relied on urine specimens collected during two narrow time windows during pregnancy. As supported by several studies including populations of adult women (Adibi, et al., 2009; Irvin, et al., 2010; Mahalingaiah, et al., 2008; Peck, et al., 2009), we have assumed that these limited serial measurements can reflect a typical exposure at any time during pregnancy. However, exposure misclassification is possible because there is considerable variability in the urinary concentrations of phthalates (Preau Jr, et al., 2010) and potentially BPA. Finally, while we have attempted to limit the dangers of multiple comparisons by only conducting full multivariable analyses on associations which were significant in bivariate analyses, we acknowledge that we have conducted a large number of tests and therefore run the risk of falsely detecting associations.

6. Conclusions

The findings of this study suggest that prenatal exposure to BPA is not associated with notable neurobehavioral outcomes during early infancy. Prenatal exposure to some phthalates was associated with greater behavioral organization but poorer reflexes in young infants. Associations varied by timing of exposure and type of phthalate. Associations were limited to exposures at the end of the second trimester of pregnancy. More favorable behavioral organization findings were found with DBP exposure, and more detrimental reflex findings were found with DEHP exposure. Additional research is necessary to further demonstrate the differential effects of some plastic components on neurobehavioral outcomes in early infancy as well as throughout childhood.

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Abbreviations

BDI	Beck Depression Inventory
BMI	body mass index
BPA	Bisphenol A
CDC	Centers for Disease Control and Prevention
DBP	di-butyl phthalate
DEHP	di-2-ethyhexyl phthalate
HOME Study	Health Outcomes and Measures of the Environment Study
LOD	limit of detection
NNNS	Neonatal Intensive Care Unit Network Neurobehavioral Scale

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Table 1

Characteristics of the Sample

Maternal Characteristics	Full Sample (N=350)
Maternal age at delivery (years)[a]	30 (5.8)
Race	
White, non-Hispanic	222 (63%)
Black, non-Hispanic	105 (30%)
Other	23 (7%)
Marital status	
Married	233 (66%)
Not married, living with someone	48 (14%)
Not married, living alone	69 (20%)
Household income[b]	55K (27K, 85K)
Employed	288 (82%)
Education	
<= HS or GED	74 (21%)
Some college or college graduate	198 (57%)
Graduate or professional school	78 (22%)
Moderate to severe depression (during pregnancy or postpartum)	89 (25%)
Alcohol use during pregnancy	
Never drank alcohol during pregnancy	192 (55%)
Drank <1 alcoholic drink per month	107 (30%)
Drank >1 alcoholic drink per month	51 (15%)
Marijuana use during pregnancy	25 (7%)
Max blood lead (microg/dL, geomean)[c]	0.82 (0.79 – 0.86)
Max serum cotinine (ng/mL, geomean)[c]	0.098 (0.074, 0.13)

Infant Characteristics	
Male	163 (46%)
Birth weight (grams)[a]	3389 (615)
Birth length (cms)[a]	50.9 (2.9)
Head circumference (cms)[a]	34.2 (1.9)
Gestational age (weeks)[a]	39.0 (1.7)
Age at 5-week exam (days)[a]	34.5 (5.0)
Birth order	
First child	153 (44%)
Second child	114 (32%)
> Second child	83 (24%)
At risk for neurodevelopmental deficits	43 (12%)
Preterm (<37 weeks)	31 (9%)
Low birth weight (<2500 grams)	19 (5%)

Infant Characteristics	
NICU stay	17 (5%)

NNNS Scale Scores	**(Range)**	
Attention	(1.2 - 9)	5.40 (1.39)
Arousal	(2.4 - 6)	4.8 (0.69)
Regulation	(2.9 - 7.5)	5.54 (0.79)
Handling	(0 - 1)	0.44 (0.31)
Movement Quality	(2.6 - 6.2)	4.81 (0.59)
Excitability	(0 - 9)	2.44 (2.02)
Lethargy	(0 - 12)	4.12 (1.75)
Nonoptimal Reflexes	(0 - 9)	3.96 (1.64)
Asymmetry	(0 - 5)	1.23 (1.08)
Hypotonia[d]	(0 - 1)	82 (23.4)
Stress/Abstinence	(0.02 - 0.35)	0.13 (0.05)

Data presented as N (%),

[a] Mean (SD),

[b] Median (25^{th}, 75^{th} percentile),

[c] Geometric Mean (95% confidence interval), or

[d] frequency reported for hypotonia=1.

Table 2

Phthalate Metabolite and BPA Levels in HOME Study Maternal Urinary Samples in Comparison with National Levels

Metabolite	National Levels Females 2003-04		HOME Study 16 week (n=346)			HOME Study 26 week (n=332)		
	Geomean (95%CI)		Geomean (95%CI)		% > LOD	Geomean (95%CI)		% > LOD
MBP/MnBP (ng/mL)	22.2	(21.2-23.3)	24.0	(21.2-27.1)	99.7	20.3	(17.9-23.0)	100
MiBP (ng/mL)	3.6	(3.19-3.97)	4.5	(4.0-5.2)	96.0	3.6	(3.1-4.2)	94.1
DBP sum (nmol/L)	Not	Available	134	(119-151)	99.7	113	(99-128)	100
MECPP (ng/mL)	31.9	(28.1-36.2)	38.0	(33.0-43.7)	100	29.9	(26.1-34.2)	100
MEHHP (ng/mL)	19.7	(17.4-22.2	26.9	(23.0-31.3)	99.1	20.4	(17.6-23.5)	99.1
MEOHP (ng/mL)	13.4	(11.9-15.1)	19.9	(17.1-23.2)	99.4	16.5	(14.3-19.1)	98.5
MEHP (ng/mL)	2.2	(1.92-242)	4.9	(4.2-5.7)	75.4	4.2	(3.7-4.9)	77.6
DEHP sum (nmol/L)	Not	Available	311	(269-360)	100	245	(213-281)	100
BPA (ng/mL)	2.4	(2.11-2.75)	1.8	(1.6-2.0)	90	1.7	(1.5-2.0)	90.3
Creatinine[a] (mg/dL)	119		124	(84)	100	105	(75)	100

*No National levels available for phthalate metabolite summary measures

[a]Mean (std) presented to allow comparison with available normative data (Barr, et al., 2005)

Table 3

Unadjusted Bivariate Associations between Maternal Phthalate and BPA Measures at Two Times and Early Infant Neurobehavior

	16w			26w		
NNNS Subscale	**Beta**	**SE**	**p**	**Beta**	**SE**	**p**
Attention						
DBP	0.022	0.069	0.75	0.104	0.074	0.16
DEHP	−0.024	0.045	0.60	−0.022	0.051	0.66
BPA	−0.030	0.064	0.64	−0.028	0.072	0.69
Arousal						
DBP	−0.004	0.033	0.89	−0.069	0.036	0.06
DEHP	0.009	0.022	0.67	0.030	0.025	0.24
BPA	0.025	0.030	0.41	0.008	0.032	0.80
Regulation						
DBP	−0.008	0.038	0.82	0.086	0.042	0.04
DEHP	−.0003	0.024	0.99	−0.002	0.029	0.95
BPA	−0.029	0.034	0.40	−0.030	0.038	0.43
Handling						
DBP	−0.019	0.015	0.21	−0.038	0.016	0.02
DEHP	−0.002	0.010	0.84	−0.005	0.011	0.64
BPA	0.011	0.014	0.42	−0.012	0.016	0.43
Movement Quality						
DBP	0.002	0.028	0.94	0.060	0.030	0.05
DEHP	0.026	0.018	0.15	0.032	0.021	0.13

	16w			26w		
NNNS Subscale	**Beta**	**SE**	**p**	**Beta**	**SE**	**p**
BPA	−0.010	0.025	0.70	0.009	0.028	0.74
Excitability						
DBP	0.040	0.096	0.68	−0.107	0.105	0.31
DEHP	0.037	0.063	0.56	0.042	0.073	0.57
BPA	0.016	0.088	0.86	0.072	0.095	0.44
Lethargy						
DBP	0.019	0.083	0.82	0.009	0.092	0.92
DEHP	0.016	0.055	0.77	0.041	0.064	0.52
BPA	0.052	0.076	0.49	0.106	0.083	0.20
Nonoptimal Reflexes						
DBP	−0.123	0.078	0.12	−0.064	0.085	0.45
DEHP	0.006	0.052	0.90	0.130	0.059	0.03
BPA	−0.018	0.072	0.80	−0.080	0.077	0.29
Asymmetry						
DBP	0.011	0.052	0.83	0.078	0.057	0.17
DEHP	0.045	0.034	0.19	0.036	0.040	0.36
BPA	0.001	0.048	0.98	−0.011	0.052	0.83
Hypotonia						
DBP	0.197	0.114	0.08	0.203	0.122	0.097
DEHP	0.020	0.074	0.78	−0.058	0.089	0.51
BPA	0.170	0.102	0.09	0.028	0.109	0.80
Stress/Abstinence						

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	16w			26w		
NNNS Subscale	**Beta**	**SE**	**p**	**Beta**	**SE**	**p**
DBP	0.002	0.003	0.41	−0.001	0.003	0.73
DEHP	−0.002	0.002	0.24	−0.002	0.002	0.39
BPA	0.004	0.002	0.12	0.002	0.002	0.34

Table 4

Mulitvariable Models of Associations between Maternal Phthalate and BPA Measures at Two Times and Early Infant Neurobehavior for NNNS Scales with Significant Bivariate Findings at p<.10

NNNS Subscale	Covariates Included	n	Beta	SE	p
				DBP	
Arousal – 26w		332	−0.072	0.036	**0.04**
Higher DBP exposure associated with lower (improved) arousal	Log_2 creatinine		0.105	0.052	0.04
	Age at exam		0.011	0.008	0.16
	Sex		−0.015	0.078	0.84
	Weight change		−.0002	.0001	0.07
	Drink1		−0.060	0.085	0.48
	Drink2		−0.270	0.113	0.02
	Log_2 cotinine max		0.014	0.011	0.18
Regulation – 26w		329	0.080	0.041	0.05
Higher DBP exposure associated with higher (improved) self-regulation	Log_2 creatinine		−0.109	0.060	0.07
	Age at exam		−0.001	0.009	0.91
	Sex		−0.144	0.089	0.11
	Weight change		0.0004	0.0002	0.02
	Marital status1		−0.419	0.137	0.002
	Marital status 2		−0.246	0.123	0.05
	Drink1		0.063	0.097	0.52
	Drink2		0.242	0.129	0.06
	High risk status		−0.195	0.132	0.14
Handling - 26w		325	−0.038	0.016	**0.02**
Higher DBP exposure associated with less handling needed	Log_2 creatinine		0.049	0.023	0.03
	Age at exam		0.005	0.003	0.12
	Sex		−0.030	0.034	0.39
Movement Quality – 26w		339	0.054	0.029	0.067
Higher DBP exposure associated with trend of improved movement	Log_2 creatinine		−0.100	0.043	0.02
	Age at exam		−0.010	0.005	0.02

NNNS Subscale	Covariates Included	n	Beta	SE	p
	Age at exam		−0.040	0.008	0.04
quality	Marital status1		−0.262	0.097	0.008
	Marital status 2		−0.130	0.087	0.14
	High risk status		−0.393	0.095	<.0001
Hypotonia* - 16w		346	0.186	0.114	0.10
	Log_2 creatinine		−0.037	0.166	0.82
	Age at exam		−0.035	0.026	0.17
	Sex		−0.109	0.258	0.67
Hypotonia* - 26w		332	0.197	0.122	0.11
	Log_2 creatinine		−0.167	0.175	0.35
	Age at exam		−0.36	0.027	0.18
	Sex		0.030	0.264	0.91
				DEHP	
Nonoptimal Reflexes ** **26w males**		158	0.216	0.090	**0.02**
	Log_2 creatinine		−0.018	0.140	0.90
Higher DBP exposure associated with more nonoptimal reflexes	Age at exam		−0.011	0.024	0.65
	Income		0.005	0.003	0.14
	Weight change		0.0003	0.0004	0.52
26w females		174	0.019	0.082	0.82
	Log_2 creatinine		−0.034	0.143	0.81
	Age at exam		0.016	0.028	0.58
	Income		0.007	0.004	0.05
	Weight change		0.0003	0.0005	0.52
				BPA	
Hypotonia* - 16w		346	0.179	0.104	0.09
Higher BPA exposure associated with trend of more hypotonia (reduced tone)	Log_2 creatinine		0.067	0.153	0.66
	Age at exam		−0.038	0.026	0.14

NNNS Subscale	Covariates Included	n	Beta	SE	p
	Sex		−0.118	0.261	0.65
	Black race		−0.341	0.310	027

* Logistic regression applied. Manuscript text reports odds ratio.

** Sex by phthalate interaction p-values: non-optimal reflexes and DEHP at 26 weeks p=0.04