Urinary 3-phenoxybenzoic acid (3-PBA) levels among pregnant women in Mexico City: Distribution and relationships with child neurodevelopment

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Abstract

Background—In recent years, pyrethroid pesticide use has increased in Mexico, the United States, and elsewhere, resulting in extensive human exposure. There is growing concern that pregnant women may be a particularly vulnerable population, as in utero fetal exposure during critical periods of development could adversely affect long-term neurobehavioral function.

Methods—We measured maternal urinary 3-phenoxybenzoic acid (3-PBA) concentrations during the third trimester of pregnancy as a measure of in utero pyrethroid exposure to the fetus among participants in an established Mexico City birth cohort (n=187). In a subset of mothers, we measured 3-PBA during the first, second, and third trimester (n=21) to assess variability across pregnancy. We examined associations between third trimester 3-PBA concentrations and children’s scores on the Mental Development Index (MDI) and Psychomotor Development Index (PDI) from the Bayley Scales for Infant Development (BSID-IIS) at 24 and 36 months of age.
Results—3-PBA was detected in 46% of all urine samples, with similar detection rates and geometric mean concentrations across pregnancy among the 21 participants who provided repeat samples. Participants in the medium and high 3-PBA categories (≥LOD) had lower MDI scores at 24 months compared to those in the low 3-PBA category (<LOD) after adjustment for covariates (p_{trend}=0.07), with slightly stronger associations among female children. The 3-level categorical variable for third trimester in utero 3-PBA was not associated with MDI scores at 36 months, or with PDI scores at either time point.

Conclusion—Considering the widespread agricultural and residential use of pyrethroids worldwide and the implications of cognitive and behavioral deficits, our findings indicate that additional study of in utero pyrethroid exposure and neurodevelopment in a larger study population is needed.

Keywords
pyrethroid; in utero; neurodevelopment; pesticide exposure; BSID-II

Introduction
In recent years, the use of pyrethroid- and pyrethrin-based pesticides in agricultural and residential settings has increased in Mexico and the U.S., mainly due to changes in regulations on pesticide use (DOF 2003, 2011; EPA 2013). Pyrethrins are naturally occurring pesticides found in some members of the chrysanthemum family (ATSDR 2003; EPA 2013), and modification of the pyrethrin structure has allowed chemists to develop more than 1,000 synthetic pyrethroids, an important class of broad-spectrum insecticides (ATSDR 2003). Pyrethroids are used in agriculture, forestry, horticulture, health care settings, and homes, as well as in textiles such as carpeting and clothing (ATSDR 2003; EPA 2013). Synthetic pyrethroids typically have a longer half-life in the environment and are often more toxic to insects and mammals compared to naturally occurring pyrethrins (ATSDR 2003).

Pyrethroids are acute neurotoxicants (Aldridge 1990; Bradbury and Coats 1989) that alter the normal function of insect nerve cells by modifying the kinetics of voltage-sensitive sodium channels (ATSDR 2003). This is similar to the mode of action of p, p’-dichlorodiphenyltrichloroethane (DDT), an insecticide that has been banned in North America due to concerns of human toxicity and persistence in the environment. However, whereas DDT has a biological half-life on the order of several years, pyrethroids are quickly metabolized with half-lives on the order of hours (Ratelle et al. 2015).

Exposure to pyrethroids in the general population is widespread (Barr et al. 2010) and thought to occur mainly via residues in the diet, although residue levels in crops grown according to good agricultural practice are generally low (WHO 1990). Inhalation or ingestion of contaminated household dust may be another important source of exposure, as pyrethroids have been highly detected in dust samples collected from homes, daycares, and other indoor environments (Hwang et al. 2008; Morgan 2012; Trunnelle et al. 2013). In previous studies, pyrethroids have been shown to persist for long periods in indoor environments (e.g. house dust) as a result of limited sunlight and moisture (Leng et al.)
Despite evidence of widespread pyrethroid exposure (CDC 2014), the impact of this exposure on long-term human health is unclear. Exposure among pregnant women is a particular concern, as many chemicals pass from mother to fetus through the placenta, and from mother to infant via breast milk (Landrigan and Miodovnik 2011). For example, animal and epidemiological studies have suggested that in utero and early life exposure to various pesticides may impair neurodevelopment and cognitive-behavioral function in childhood (Chanda and Pope 1996; Eskenazi et al. 2007; Julvez and Grandjean 2009; Jurewicz and Hanke 2008; Marks et al. 2010; Young et al. 2005). Animal studies indicate that pyrethroid-based pesticides could affect the developing brain due to the persistent effects on neurotransmitters (Malaviya et al. 1993; Santoni et al. 1999); however, studies of pyrethroid exposure and the related health effects among humans are lacking.

Our objectives in the present study were to characterize urinary concentrations of 3-PBA in a cohort of pregnant women in Mexico City, and to investigate relationships between prenatal 3-PBA concentrations – a marker of in utero pyrethroid exposure to the fetus – and subsequent measures of early child neurodevelopment.

**Methods**

**Study Population**

Participants in the present study were enrolled between 1997 and 2001 into the Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT) study, a longitudinal cohort study of pregnant women in Mexico City and their offspring. As previously described, women were enrolled during their first trimester of pregnancy from the National Institute of Perinatology, Hospital General Dr. Manual Gea Gonzalez, or clinics affiliated with the Mexican Social Security Institute, and participated in follow-up visits until their children were 5 years of age (Claus Henn et al. 2010). Exclusion characteristics for initial enrollment included: plans to leave the area within the next 5 years; daily consumption of alcoholic beverages during pregnancy; addiction to illegal drugs; habitual use of prescription drugs; diagnosis of high risk pregnancy, pre-eclampsia, gestational diabetes, or renal or heart disease; a history of infertility, diabetes, or psychosis; or suffering from seizures requiring medical treatment (Hu et al. 2006). Children were excluded from neurodevelopmental assessments if they were very low birth weight (<1.5 kg) or born severely premature (<32 weeks gestation) (Claus Henn et al. 2010). The Institutional Review Boards of the National Institute of Public Health (Mexico), Harvard School of Public Health, and participating hospitals approved all study materials and procedures. Participants were informed of the study, associated aims, and uses of biological samples/data and written consent was obtained before enrollment.

In the overall ELEMENT study, participants provided second morning void urine samples during each trimester of pregnancy. In the current study, we measured 3-PBA concentrations...
in third trimester urine samples from mothers whose children had completed psychometric assessments at age 2 and 3 years (n=187). In a random subset of women (n=21) selected from the 187 mother-child pairs, we measured 3-PBA in urine samples collected during each trimester to examine variability in repeat samples over the course of pregnancy.

**Assessment of Early Childhood Neurodevelopment**

We used the Bayley Scales for Infant Development—Spanish version (BSID-II) to assess developmental functioning of infants and children at 24 and 36 months of age using the Mental Development Index (MDI) and Psychomotor Development Index (PDI). The BSID-II is designed to identify young children with developmental delay (i.e. cognitive, language, personal-social, fine and gross motor development) and to provide information for intervention planning for children between the ages of 1 and 42 months (Bayley 1993). Each index has a mean score of 100 and SD of 15. Children with scores of <70 are classified as significantly delayed. Research personnel who administered the assessments were trained and supervised by an expert member of our research team (L.S.). Standardization and quality control checks were conducted by reviews of videotaped evaluations.

**Measurement of Urinary 3-PBA Concentrations**

Maternal urine samples (2 mL) were transported on dry ice to Emory University for analysis of 3-PBA using previously described methods (Olsson et al. 2004). Samples were spiked with stable isotopically labeled 3-PBA and subjected to enzyme hydrolysis. Hydrolysates were extracted using mixed-polarity solid-phase extraction cartridges and eluates were concentrated and analyzed using high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) with both quantification and confirmation ions monitored. A matrix based isotope dilution calibration was used for quantification (Olsson et al. 2004). The limit of detection (LOD) for urinary 3-PBA was 0.25 ng/mL. To calculate the geometric mean and 3-PBA distributions, values below the LOD were assigned a value of LOD/√2 (Hornung and Reed 1990).

**Data Analysis**

Urinary 3-PBA concentrations were below the limit of detection for a large number of urine samples, so we grouped exposure into two categories for descriptive analyses (detected vs. not detected) and into three categories for regression analyses. The lowest category of the three level variable comprised all samples below the LOD (n=103); the medium and high categories were formed by dividing the samples with detectable 3-PBA into two equally sized groups (n=42 each).

We used chi-square and t-tests to determine differences in categorical and continuous demographic characteristics between mothers who had detectable levels of urinary 3-PBA and those that did not. We used linear regression to investigate associations between the categorical urinary 3-PBA measure (low, medium and high) and developmental assessment scores measured at 24 and 36 months (MDI-24, MDI-36, PDI-24, and PDI-36). Maternal education (years), IQ score, socioeconomic status (SES) score, blood lead, and child sex were included in multivariable models as potential confounders, and urinary specific gravity was included as a covariate to adjust for urine dilution. Maternal IQ was calculated based on
scores on the Information, Comprehension, Similarities, and Block Designs scales of the Spanish Wechsler Adult Intelligence Scale (Hu et al. 2006; Wechsler 1968). As a surrogate measure of SES during early pregnancy and early childhood, we used questionnaire information collected in 2007–2011 during a subsequent follow-up study of the same study participants. With this information, we created a continuous SES score as previously described (Fortenberry et al. 2014). Briefly, participants were asked questions about their household, including the number of light bulbs, rooms, and bathrooms in their home, the number of cars they owned, whether they owned a personal computer, water heater, and/or electrical appliances, and the type of house floor, as recommended by the Asociación Mexicana de Agencias de Investigación (AMAI) (AMAI 2005). Each item was assigned a point value and then summed for the total SES score, with higher scores indicating higher economic status. Maternal blood lead concentrations were measured 1 month after delivery as a measure of in utero and lactational lead exposure to their child (Ettinger et al. 2004; Ettinger et al. 2014). Linear trend estimates for the categorical 3-PBA measure were calculated by entering the 3-level ordinal exposure variable into regression models as a continuous variable. In order to explore sex-specific relationships between measures of exposure and neurodevelopment, we ran models with all children combined and stratified by sex. We used Statistical Analysis Software (SAS) (version 9.4; SAS Institute Inc., Cary, NC, USA) for all analyses.

Results

Participant Characteristics and Exposure

Demographic characteristics of women in the current analysis are presented in Table 1. The median maternal age at delivery was 26 years, with 11 years of schooling and a median IQ of 96. Most mothers were married or living with a partner (91%), did not smoke during pregnancy (99%), breastfed their baby at some point during infancy (90%), and 48% of their infants in the current analysis were male. Median maternal blood lead concentrations at 1 month after delivery (5.4 μg/dl) were slightly above the upper acceptable limit (5.0 μg/dL) recommended by the CDC for pregnant and lactating women (CDC 2010). Mothers with detectable levels of 3-PBA in their urine were older (p=0.002), slightly less educated (p=0.04), and had lower SES scores (p=0.05), than mothers without detectable 3-PBA. Among child participants, mean MDI and PDI scores at 24 and 36 months were slightly below 100 (Table 2), the standardized mean score of a representative U.S. population (Bayley 1993).

A total of 229 urine samples were analyzed for 3-PBA. These samples consisted of 187 third trimester samples from women who had children with data on at least one of the neurodevelopmental outcome measures of interest. The remaining samples were from the subset of women (n=21) for whom we also measured urinary 3-PBA during their first and second trimesters. 3-PBA was detected in 46% of all urine samples and 45% of third trimester samples (Table 3). Detection rates and geometric mean 3-PBA concentrations were similar during the first, second, and third trimesters of pregnancy among the 21 participants who provided urine samples at all three trimesters, although concentrations at the 75th and 95th percentiles of exposure did appear to decrease over pregnancy.
The median urinary 3-PBA concentration within our study population was <0.25 ng/mL, while the median concentration within pregnant women who participated in the National Health and Nutrition Examination Survey (NHANES) during the same time period (n=205) was 0.23 ng/mL (below our LOD) (Table 3). However, the 75th and 95th percentiles in the NHANES population (0.46 and 2.19 ng/mL) were higher than the same percentiles in the current study (0.34 and 0.84 ng/mL).

In Utero Pyrethroid Exposure and Early Childhood Neurodevelopment

We observed lower MDI scores at 24 months among participants in the medium and high 3-PBA categories compared to those with non-detectable urinary 3-PBA (−3.5, −3.8 respectively; \( P_{\text{trend}} = 0.07 \)) after adjustment for maternal IQ, education, SES score, blood lead, urinary specific gravity, and the sex of the child (Table 4). These findings were slightly stronger among girls, with MDI-24 scores among those in the medium and high 3-PDA groups 6 points below scores of girls in the non-detect category (95% CI = −12.3, −0.14 and −12.4, 1.3, respectively; \( P_{\text{trend}} = 0.08 \)) after adjustment for covariates. At 36 months, scores among children in the high 3-PBA category were still lower than those among children with undetectable levels of 3-PBA, although these findings were not statistically significant (\( P_{\text{trend}} = 0.14 \); Table 4). We did not observe associations between maternal 3-PBA levels during the third trimester of pregnancy and PDI scores at 24 or 36 months of age among all participants or in sex-stratified analyses (Table 5).

Discussion

Few studies have evaluated relationships between in utero exposure to pyrethroid-based pesticides and neurodevelopment in humans, despite evidence from animal studies that exposure during this potential window of vulnerability may have deleterious effects. We observed suggestive associations between maternal urinary 3-PBA levels during the third trimester of pregnancy, a marker of in utero pyrethroid exposure to their child, and lower mental development assessment scores at 2 years of age.

Characterization of Pyrethroid Exposure

A number of previous studies have measured 3-PBA and other metabolites in urine samples collected from both adults and children to assess pyrethroid exposure (Barr et al. 2010; Fortin et al. 2008; Heudorf and Angerer 2001; Lu et al. 2006; Qi et al. 2012). We found that 3-PBA levels in this urban Mexican population were similar to levels measured in pregnant women who participated in the NHANES during the same time period. However, the higher LOD in our 3-PBA analysis (0.25 vs. 0.10 ng/mL) somewhat limits our ability to make direct comparisons. In addition, 3-PBA concentrations were measured during all trimesters of pregnancy among NHANES participants, while we measured 3-PBA primarily during the third trimester.

In a study of pregnant women (n=1149) living in Jiangsu Province, an agricultural area in China, the geometric mean 3-PBA concentration was 0.97 ng/mL (unadjusted for specific gravity) (Qi et al. 2012). Children from agricultural regions of Nicaragua and Thailand have also been shown to have relatively high pyrethroid exposures (Panuwet et al. 2009;
Rodriguez et al. 2012). In a study of 205 children in Thailand, children of agricultural families had significantly higher geometric mean 3-PBA concentrations (0.38 ng/mL) compared to children of non-agricultural families (0.15 ng/mL) (Panuwet et al. 2009). Urinary 3-PBA concentrations among women in our study were lower than concentrations measured in a cohort of pregnant women in New York City during the same time period (Berkowitz et al. 2003). In addition, a 2008–2011 study of pregnant women in Caribbean countries also observed higher urinary 3-PBA concentrations compared to our study population (Dewailly et al. 2014), although changes in pyrethroid use may have occurred in the time period between studies. Generally, urinary 3-PBA concentrations within our study population were consistent with those among adults in German studies, where median values of 3-PBA ranged from 0.04 ng/mL to 0.29 ng/mL (Becker et al. 2006; Heudorf et al. 2006), and a study in Quebec, Canada, which had a median of 0.17 ng/mL (Fortin et al. 2008).

To our knowledge, there are no previous studies examining variability in 3-PBA concentrations measured in repeat urine samples collected throughout pregnancy. The consistency of 3-PBA concentrations over time may be influenced by seasonal variation and by short term variations in exposure, as pyrethroid parent compounds are rapidly metabolized in humans (Ratelle et al. 2015). Unfortunately, our limit of detection for 3-PBA in urine samples was relatively high in comparison to concentrations within our study population, so over half of our samples had values below the LOD. This greatly limited our ability to formally test variability of urinary 3-PBA levels over time within individuals and across pregnancy. Nevertheless, detection rates and geometric mean concentrations in samples collected during the first, second, and third trimesters were quite similar, suggesting similar average levels of exposure throughout pregnancy. Concentrations among those at the 75th and 95th percentiles of exposure did appear to decrease during pregnancy, however we were not able to formally test this trend.

Pyrethroid Exposure and Neurodevelopment

In the present study, children in the medium and high categories of in utero pyrethroid exposure had lower MDI scores at 24 months among compared to participants in the lowest category of exposure, although this finding was only marginally significant. This may be at least partially due to our small sample size, as effect estimates from crude models were similar in magnitude and statistically significant (data not shown). Interestingly, effect estimates varied by sex, with stronger negative associations seen in girls compared to boys. Future studies of in utero and early life pyrethroid exposure and neurodevelopment should also investigate sex-specific effects, as this finding may provide insight into potential mechanisms of action.

We did not observe associations between in utero 3-PBA concentrations and PDI scores at either 24 or 36 months of age, suggesting that prenatal pyrethroid exposure may have an impact on cognitive rather than motor development. Although associations between in utero 3-PBA and MDI in the current study were modest, this finding is of concern because MDI scores are predictive of school readiness (Patrianakos-Hoobler et al. 2010), and are a possible indicator of subsequent neurodevelopment (Luttikhuizen dos Santos et al. 2013; Mazer et al. 2010).
In a previous study in New York City, researchers did not find an association between permethrin, a pyrethroid metabolized to 3-PBA, in maternal or cord plasma samples and BSID-II scores at 36 months of age among 348 mother-infant pairs (Horton et al. 2011). However, researchers did report an association between piperonyl butoxide, a common pyrethroid synergist, and increased odds of delayed mental development. Similarly, researchers in France recently reported that among 287 mother-infant pairs, maternal urinary pyrethroid metabolite concentrations during in utero development were not associated with cognitive function at age 6 years, although 3-PBA concentrations in urine collected from children at the time of neurocognitive testing were associated with decreased verbal comprehension and working memory (Viel et al. 2015). Our results are consistent with findings from these studies, as we observed associations between in utero pyrethrin exposure and neurodevelopment at 24 months, but not in older children at 36 months of age.

A recent study of 6–8 year olds in Thailand reported a cross-sectional relationship between urinary 3-PBA concentrations and lower measures of learning during the low pesticide use season, but not during the season of expected high pesticide use. In addition, average 3-PBA concentrations across seasons and the within-person change in 3-PBA concentrations across seasons were not associated with measures of neurobehavioral function (Fiedler et al. 2015). Two large cross-sectional studies of pyrethroid exposure and neurobehavioral outcomes in children aged 6–15 years did not report a significant relationship between 3-PBA and neurobehavior (Oulhote and Bouchard 2013; Quiros-Alcala et al. 2014). However, one of these studies did report increased odds of parent-reported behavioral problems in relation to urinary cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (cis-DCCA), another common pyrethroid metabolite (Oulhote and Bouchard 2013). For comparison, cis- and trans-DCCA were not detected in maternal urine samples collected in the current study (data not shown).

**Strengths and Limitations**

Our study had a number of strengths and limitations. Limitations include a relatively small sample size for exploring relationships between urinary 3-PBA and childhood neurodevelopment and the use of a single urinary measure to estimate exposure in those analyses. Despite our modest sample size, our results do provide justification for further study. Also, 54% of all 3-PBA measurements were below the limit of detection, limiting our ability to assess linear relationships between continuous measures of exposure and outcome, as well as variability within individuals over time. Strengths of our study included the use of urinary 3-PBA as a biomarker of exposure and the use of validated neurodevelopment assessments administered by a trained and experienced research team. Using urinary 3-PBA as a biomarker of exposure to several pyrethroids provides a comprehensive exposure measure for this class of insecticides, although it does limit our ability to determine which specific pyrethroids may be more or less toxic based on our findings. However, urinary measures of pyrethroids are likely to be more reliable over time in comparison to parent compounds in blood given the rapid metabolism and low detection rates of non-persistent pesticides in blood.
In summary, these results are important considering the continued widespread agricultural and residential use of pyrethroids worldwide (EPA 2013; Tillett 2012) and the educational implications of cognitive and behavioral deficits. Relationships between pyrethroid exposure during critical periods of development and subsequent neurobehavioral function are still unclear, but these relationships deserve further study in a larger population.

Acknowledgments

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Abbreviations

3-PBA 3-phenoxybenzoic acid
AMAI Asociación Mexicana de Agencias de Investigación
BSID-IIS Bayley Scales for Infant Development—2nd edition, Spanish version
cis-DCCA cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid
DDT p, p′-dichlorodiphenyltrichloroethane
ELEMENT Early Life Exposures in Mexico to Environmental Toxicants
LOD limit of detection
MDI Mental Development Index
NHANES National Health and Nutrition Examination Survey
PDI Psychomotor Development Index
SES socioeconomic status

References

Centers for Disease Control and Prevention (CDC). Guidelines for the identification and management of lead exposure in pregnant and lactating women. 2010
Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for pyrethrins and pyrethroids. 2003


Tillett T. Optimizing insecticide resources: Global trends in vector control. Environ Health Persp. 2012; 120:A164.


Wechsler, H. Wechsler Adult Intelligence Scale (W AIS), Spanish version. San Antonio, TX: Psychological Corporation; 1968.


Highlights

- Pyrethroid use has increased worldwide, resulting in extensive human exposure.
- We examined in utero 3-PBA levels in relation to BSID-IIS scores at 2 and 3 years.
- Participants with 3-PBA ≥ LOD had lower MDI scores at 2 years compared to those < LOD.
- In utero 3-PBA levels were not associated with PDI scores at 2 or 3 years.
- Additional study of in utero pyrethroid exposure and neurodevelopment is needed.
Table 1

Demographic characteristics of participating mothers overall and stratified by 3-PBA detection in maternal urine samples collected in the third trimester of pregnancy.

<table>
<thead>
<tr>
<th>Maternal Characteristics</th>
<th>All</th>
<th></th>
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<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Median</td>
<td>(25th, 75th)</td>
<td>N</td>
<td>Median</td>
<td>(25th, 75th)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>187</td>
<td>26</td>
<td>(22, 30)</td>
<td>84</td>
<td>28 **</td>
<td>(23, 33)</td>
</tr>
<tr>
<td>Education (years)</td>
<td>187</td>
<td>11</td>
<td>(9, 12)</td>
<td>84</td>
<td>11 *</td>
<td>(9, 12)</td>
</tr>
<tr>
<td>IQ Score</td>
<td>187</td>
<td>96</td>
<td>(88, 103)</td>
<td>84</td>
<td>94</td>
<td>(86, 102)</td>
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<tr>
<td>SES Score</td>
<td>178</td>
<td>8</td>
<td>(6, 10.5)</td>
<td>77</td>
<td>6</td>
<td>(6, 10)</td>
</tr>
<tr>
<td>Blood Lead (μg/dL)</td>
<td>187</td>
<td>5.4</td>
<td>(3.3, 7.8)</td>
<td>84</td>
<td>5.5</td>
<td>(3.4, 8.2)</td>
</tr>
<tr>
<td>Urinary Specific Gravity</td>
<td>187</td>
<td>1.013</td>
<td>(1.009, 1.017)</td>
<td>84</td>
<td>1.016 **</td>
<td>(1.012, 1.019)</td>
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</tbody>
</table>

* p<0.05,
** p<0.01
Table 2

Distribution of Bayley Scales for Infant Development—Spanish version (BSID-IIS) scores among child participants at 24 and 36 months of age.

<table>
<thead>
<tr>
<th>Development Index</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Min</th>
<th>25th</th>
<th>50th</th>
<th>75th</th>
<th>Max</th>
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<tr>
<td>MDI – 24 months</td>
<td>181</td>
<td>87.1</td>
<td>11.6</td>
<td>56</td>
<td>80</td>
<td>86</td>
<td>94</td>
<td>122</td>
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<tr>
<td>MDI – 36 months</td>
<td>169</td>
<td>91.3</td>
<td>9.50</td>
<td>71</td>
<td>84</td>
<td>92</td>
<td>98</td>
<td>120</td>
</tr>
<tr>
<td>PDI – 24 months</td>
<td>180</td>
<td>92.6</td>
<td>9.89</td>
<td>61</td>
<td>84.5</td>
<td>92</td>
<td>100</td>
<td>117</td>
</tr>
<tr>
<td>PDI – 36 months</td>
<td>169</td>
<td>94.2</td>
<td>12.0</td>
<td>70</td>
<td>85</td>
<td>92</td>
<td>104</td>
<td>128</td>
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</tbody>
</table>

SD-standard deviation; MDI-Mental Development Index; PDI-Psychomotor Development Index
### Table 3

Distribution of urinary 3-PBA concentrations (ng/mL) among ELEMENT mothers throughout pregnancy, and in pregnant women participating in the 1999–2002 NHANES.

<table>
<thead>
<tr>
<th></th>
<th>Selected Percentiles</th>
<th></th>
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<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>% &lt;LOD</td>
<td>GM</td>
<td>GSD</td>
<td>25th</td>
<td>50th</td>
<td>75th</td>
<td>95th</td>
<td>Max</td>
</tr>
<tr>
<td>ELEMENT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All samples</td>
<td>229</td>
<td>53.7</td>
<td>0.26</td>
<td>1.80</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>0.34</td>
<td>0.84</td>
<td>11.1</td>
</tr>
<tr>
<td>First Trimester</td>
<td>21</td>
<td>47.6</td>
<td>0.27</td>
<td>1.65</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>0.25</td>
<td>0.38</td>
<td>0.65</td>
</tr>
<tr>
<td>Second Trimester</td>
<td>21</td>
<td>42.9</td>
<td>0.26</td>
<td>1.47</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>0.25</td>
<td>0.35</td>
<td>0.43</td>
</tr>
<tr>
<td>Third Trimester</td>
<td>21</td>
<td>57.1</td>
<td>0.23</td>
<td>1.43</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>0.29</td>
<td>0.35</td>
<td>0.61</td>
</tr>
<tr>
<td>Third Trimester</td>
<td>187</td>
<td>55.1</td>
<td>0.26</td>
<td>1.85</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>0.34</td>
<td>0.85</td>
<td>11.1</td>
</tr>
<tr>
<td>NHANES **</td>
<td>205</td>
<td>31.7</td>
<td>0.24</td>
<td>3.37</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>0.23</td>
<td>0.46</td>
<td>2.19</td>
</tr>
</tbody>
</table>

GM—geometric mean; GSD—geometric standard deviation; LOD—limit of detection: ELEMENT 3-PBA=0.25 ng/mL and NHANES 3-PBA=0.1 ng/mL.

* Among women with measured urinary 3-PBA concentrations from all three trimesters of pregnancy.

Table 4

Associations between maternal urinary 3-PBA concentrations during the third trimester of pregnancy and child MDI scores at 24 and 36 months of age, overall and stratified by child sex. Models were adjusted for urinary specific gravity, maternal IQ, education, SES score, blood lead, and child sex (except for sex-stratified models).

<table>
<thead>
<tr>
<th></th>
<th>MDI 24</th>
<th></th>
<th>MDI 36</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>β</td>
<td>p-value</td>
<td>(95% CI)</td>
</tr>
<tr>
<td>All Participants</td>
<td>172</td>
<td>−3.80</td>
<td>0.11</td>
<td>(−8.44, 0.84)</td>
</tr>
<tr>
<td>High 3-PBA</td>
<td>37</td>
<td>−3.54</td>
<td>0.11</td>
<td>(−7.86, 0.78)</td>
</tr>
<tr>
<td>Med 3-PBA</td>
<td>38</td>
<td>−3.54</td>
<td>0.11</td>
<td>(−7.86, 0.78)</td>
</tr>
<tr>
<td>3-PBA &lt; LOD</td>
<td>97 ref</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p for trend</td>
<td>0.07</td>
<td></td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>84</td>
<td>−3.43</td>
<td>0.31</td>
<td>(−10.2, 3.30)</td>
</tr>
<tr>
<td>High 3-PBA</td>
<td>17</td>
<td>−3.43</td>
<td>0.31</td>
<td>(−10.2, 3.30)</td>
</tr>
<tr>
<td>Med 3-PBA</td>
<td>15</td>
<td>−2.58</td>
<td>0.43</td>
<td>(−9.10, 3.94)</td>
</tr>
<tr>
<td>3-PBA &lt; LOD</td>
<td>52 ref</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p for trend</td>
<td>0.26</td>
<td></td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>88</td>
<td>−5.54</td>
<td>0.11</td>
<td>(−12.4, −0.34)</td>
</tr>
<tr>
<td>High 3-PBA</td>
<td>20</td>
<td>−5.54</td>
<td>0.11</td>
<td>(−12.4, −0.34)</td>
</tr>
<tr>
<td>Med 3-PBA</td>
<td>23</td>
<td>−6.20</td>
<td>0.05</td>
<td>(−12.3, −0.14)</td>
</tr>
<tr>
<td>3-PBA &lt; LOD</td>
<td>45 ref</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p for trend</td>
<td>0.08</td>
<td></td>
<td>0.24</td>
<td></td>
</tr>
</tbody>
</table>
Table 5

Associations between maternal urinary 3-PBA concentrations during the third trimester of pregnancy and child PDI scores at 24 and 36 months of age, overall and stratified by child sex. Models were adjusted for urinary specific gravity, maternal IQ, education, SES score, blood lead, and child sex (except for sex-stratified models).

<table>
<thead>
<tr>
<th></th>
<th>PDI 24</th>
<th></th>
<th>PDI 36</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>β</td>
<td>p-value</td>
<td>(95% CI)</td>
</tr>
<tr>
<td>All Participants</td>
<td>171</td>
<td>-0.42</td>
<td>0.84</td>
<td>(-4.55, 3.70)</td>
</tr>
<tr>
<td>High 3-PBA</td>
<td>36</td>
<td>-0.42</td>
<td>0.84</td>
<td>(-4.55, 3.70)</td>
</tr>
<tr>
<td>Med 3-PBA</td>
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<td>0.64</td>
<td>0.74</td>
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</tr>
<tr>
<td>3-PBA &lt; LOD</td>
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<tr>
<td>p for trend</td>
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</tr>
<tr>
<td>Male</td>
<td>83</td>
<td>-0.59</td>
<td>0.85</td>
<td>(-6.72, 5.53)</td>
</tr>
<tr>
<td>High 3-PBA</td>
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<td>-0.59</td>
<td>0.85</td>
<td>(-6.72, 5.53)</td>
</tr>
<tr>
<td>Med 3-PBA</td>
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<td>2.95</td>
<td>0.32</td>
<td>(-2.93, 8.83)</td>
</tr>
<tr>
<td>3-PBA &lt; LOD</td>
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<td>0.92</td>
<td>0.44</td>
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</tr>
<tr>
<td>p for trend</td>
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</tr>
<tr>
<td>Female</td>
<td>88</td>
<td>-3.32</td>
<td>0.25</td>
<td>(-9.04, 2.40)</td>
</tr>
<tr>
<td>High 3-PBA</td>
<td>20</td>
<td>-3.32</td>
<td>0.25</td>
<td>(-9.04, 2.40)</td>
</tr>
<tr>
<td>Med 3-PBA</td>
<td>23</td>
<td>-3.14</td>
<td>0.22</td>
<td>(-8.21, 1.94)</td>
</tr>
<tr>
<td>3-PBA &lt; LOD</td>
<td>45 ref</td>
<td>0.21</td>
<td>0.91</td>
<td></td>
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<tr>
<td>p for trend</td>
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</tbody>
</table>