Published in final edited form as:

Environ Res. 2016 May ; 147: 307–313. doi:10.1016/j.envres.2016.02.025.

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

Deborah J. Watkins^a, Gamola Z. Fortenberry^a, Brisa N. Sánchez^b, Dana Boyd Barr^c, Parinya Panuwet^c, Lourdes Schnaas^d, Erika Osorio-Valencia^d, Maritsa Solano-González^e, Adrienne S. Ettinger^f, Mauricio Hernández-Ávila^e, Howard Hu^g, Martha María Téllez-Rojo^e, and John D. Meeker^{a,*}

^aDepartment of Environmental Health Sciences, School of Public Health, University of Michigan, Ann Arbor, MI, USA

^bDepartment of Biostatistics, School of Public Health, University of Michigan, Ann Arbor, MI, USA

^cDepartment of Environmental Health, Emory University, Atlanta, GA, USA

^dDivision of Research on Public Health, National Institute of Perinatology, Mexico City, Mexico

^eNational Institute of Public Health, Cuernavaca, Morelos, Mexico

^fDepartment of Chronic Disease Epidemiology, Yale School of Public Health, New Haven, CT, USA

^gDalla Lana School of Public Health, University of Toronto, ON, Canada

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.

*Corresponding author: John D. Meeker, meekerj@umich.edu, Phone: +1 (734) 764-7184, Address: Department of Environmental Health Sciences, University of Michigan School of Public Health, Room 1835 SPH I, 1415 Washington Heights, Ann Arbor, MI, 48109-2029.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

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 ($p_{\text{trend}}=0.07$), 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.

2005), and urinary concentrations of 3-phenoxybenzoic acid (3-PBA), a non-specific metabolite of several pyrethroids and biomarker of exposure, have been associated with pyrethroid levels in house dust (Heudorf and Angerer 2001; Morgan et al. 2007; Trunnelle et al. 2014).

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. Manuel 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-IIS) 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-PBA 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 (Luttikhuisen 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

This work was supported by the National Institute of Environmental Health Sciences grant numbers: R01ES021446, 01ES007821, R01ES021465, P42ES017198, P01ES022844, P30ES017885, and P30ES019776; by the U.S. Environmental Protection Agency, grant number:R835436; by the National Institute of Public Health (INSP)/Ministry of Health of Mexico; by Consejo Nacional De Ciencia Y Tecnología (CONACyT) grant number: 4150M9405; and by the Consejo de Estudios para La Restauración y Valoración Ambiental (CONSERVA), Department of Federal District, México.

Abbreviations

3-PBA	3-phenoxybenzoic acid
AMAI	Asociación Mexicana de Agencias de Investigación
BSID-IIS	Bayley Scales for Infant Development—2 nd edition, Spanish version
cis-DCCA	<i>cis</i> -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

- Asociación Mexicana de Agencias de Investigación (AMAI). [accessed September 1 2015] AMAI advances: Distribution of socioeconomic levels in urban Mexico. 2005. Available: https://www.cgidd.com/socioeconomic_definition.pdf
- Centers for Disease Control and Prevention (CDC). Guidelines for the identification and management of lead exposure in pregnant and lactating women. 2010
- Centers for Disease Control and Prevention (CDC). Fourth national report on human exposure to environmental chemicals. Atlanta, GA: 2014. Updated tables, July 2014
- Aldridge WN. An assessment of the toxicological properties of pyrethroids and their neurotoxicity. *Crit Rev Toxicol*. 1990; 21:89–104. [PubMed: 2083034]
- Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for pyrethrins and pyrethroids. 2003
- Barr DB, Olsson AO, Wong LY, Udunka S, Baker SE, Whitehead RD, et al. Urinary concentrations of metabolites of pyrethroid insecticides in the general U.S. Population: National health and nutrition examination survey 1999–2002. *Environ Health Persp*. 2010; 118:742–748.
- Bayley, N. Bayley scales of infant development - second edition. San Antonio, TX: The Psychological Corporation; 1993.

- Becker K, Seiwert M, Angerer J, Kolossa-Gehring M, Hoppe HW, Ball M, et al. GerES IV pilot study: Assessment of the exposure of German children to organophosphorus and pyrethroid pesticides. *Int J Hyg Envir Heal*. 2006; 209:221–233.
- Berkowitz GS, Obel J, Deych E, Lapinski R, Godbold J, Liu Z, et al. Exposure to indoor pesticides during pregnancy in a multiethnic, urban cohort. *Environ Health Persp*. 2003; 111:79–84.
- Bradbury SP, Coats JR. Comparative toxicology of the pyrethroid insecticides. *Rev Environl Contam T*. 1989; 108:133–177.
- Chanda SM, Pope CN. Neurochemical and neurobehavioral effects of repeated gestational exposure to chlorpyrifos in maternal and developing rats. *Pharmacol Biochem Be*. 1996; 53:771–776.
- Claus Henn B, Ettinger AS, Schwartz J, Tellez-Rojo MM, Lamadrid-Figueroa H, Hernandez-Avila M, et al. Early postnatal blood manganese levels and children's neurodevelopment. *Epidemiology*. 2010; 21:433–439. [PubMed: 20549838]
- Dewailly E, Forde M, Robertson L, Kaddar N, Laouan Sidi EA, Cote S, et al. Evaluation of pyrethroid exposures in pregnant women from 10 Caribbean countries. *Environ Int*. 2014; 63:201–206. [PubMed: 24317226]
- Diario Oficial de la Federacion (DOF). Prevention and control of vector-transmitted diseases. Mexico Fed Reg NOM-032-SSA-2-2002 for epidemiological monitoring. 2003
- Diario Oficial de la Federacion (DOF). Prevention and control of vector-transmitted diseases. Mexico Fed Reg NOM-EM-003-SSA2-2010 for epidemiological monitoring. 2011
- Environmental Protection Agency (EPA). [accessed May 19, 2015] Pyrethroids and pyrethrins. 2013. Available: <http://www.epa.gov/oppsrrd1/reevaluation/pyrethroids-pyrethrins.html>
- Eskenazi B, Marks AR, Bradman A, Harley K, Barr DB, Johnson C, et al. Organophosphate pesticide exposure and neurodevelopment in young Mexican-American children. *Environ Health Persp*. 2007; 115:792–798.
- Ettinger AS, Tellez-Rojo MM, Amarasiriwardena C, Bellinger D, Peterson K, Schwartz J, et al. Effect of breast milk lead on infant blood lead levels at 1 month of age. *Environ Health Persp*. 2004; 112:1381–1385.
- Ettinger AS, Roy A, Amarasiriwardena CJ, Smith D, Lupoli N, Mercado-Garcia A, et al. Maternal blood, plasma, and breast milk lead: Lactational transfer and contribution to infant exposure. *Environ Health Persp*. 2014; 122:87–92.
- Fiedler N, Rohitattana J, Siri Wong W, Suttiwan P, Ohman Strickland P, Ryan PB, et al. Neurobehavioral effects of exposure to organophosphates and pyrethroid pesticides among Thai children. *Neurotoxicology*. 2015; 48:90–99. [PubMed: 25721160]
- Fortenberry GZ, Meeker JD, Sanchez BN, Bellinger D, Peterson K, Schnaas L, et al. Paraoxonase I polymorphisms and attention/hyperactivity in school-age children from Mexico City, Mexico. *Environ Res*. 2014; 132:342–349. [PubMed: 24834831]
- Fortin MC, Bouchard M, Carrier G, Dumas P. Biological monitoring of exposure to pyrethrins and pyrethroids in a metropolitan population of the province of Quebec, Canada. *Environ Res*. 2008; 107:343–350. [PubMed: 18436207]
- Heudorf U, Angerer J. Metabolites of pyrethroid insecticides in urine specimens: Current exposure in an urban population in Germany. *Environ Health Persp*. 2001; 109:213–217.
- Heudorf U, Butte W, Schulz C, Angerer J. Reference values for metabolites of pyrethroid and organophosphorous insecticides in urine for human biomonitoring in environmental medicine. *Int J Hyg Envir Heal*. 2006; 209:293–299.
- Hornung RW, Reed LD. Estimation of average concentration in the presence of nondetectable values. *Appl Occup Environ Hyg*. 1990; 5:46–51.
- Horton MK, Rundle A, Camann DE, Boyd Barr D, Rauh VA, Whyatt RM. Impact of prenatal exposure to piperonyl butoxide and permethrin on 36-month neurodevelopment. *Pediatrics*. 2011; 127:e699–706. [PubMed: 21300677]
- Hu H, Tellez-Rojo MM, Bellinger D, Smith D, Ettinger AS, Lamadrid-Figueroa H, et al. Fetal lead exposure at each stage of pregnancy as a predictor of infant mental development. *Environ Health Persp*. 2006; 114:1730–1735.
- Hwang HM, Park EK, Young TM, Hammock BD. Occurrence of endocrine-disrupting chemicals in indoor dust. *Sci Total Environ*. 2008; 404:26–35. [PubMed: 18632138]

- Julvez J, Grandjean P. Neurodevelopmental toxicity risks due to occupational exposure to industrial chemicals during pregnancy. *Ind Health*. 2009; 47:459–468. [PubMed: 19834254]
- Jurewicz J, Hanke W. Prenatal and childhood exposure to pesticides and neurobehavioral development: Review of epidemiological studies. *Int J Occup Med Env*. 2008; 21:121–132.
- Landrigan PJ, Miodovnik A. Children's health and the environment: An overview. *Mt Sinai J Med*, New York. 2011; 78:1–10.
- Leng G, Berger-Preiss E, Levsen K, Ranft U, Sugiri D, Hadnagy W, et al. Pyrethroids used indoor-ambient monitoring of pyrethroids following a pest control operation. *Int J Hyg Envir Heal*. 2005; 208:193–199.
- Lu C, Barr DB, Pearson M, Bartell S, Bravo R. A longitudinal approach to assessing urban and suburban children's exposure to pyrethroid pesticides. *Environ Health Persp*. 2006; 114:1419–1423.
- Luttikhuisen dos Santos ES, de Kieviet JF, Konigs M, van Elburg RM, Oosterlaan J. Predictive value of the Bayley Scales of Infant Development on development of very preterm/very low birth weight children: A meta-analysis. *Early Hum Dev*. 2013; 89:487–496. [PubMed: 23597678]
- Malaviya M, Husain R, Seth PK, Husain R. Perinatal effects of two pyrethroid insecticides on brain neurotransmitter function in the neonatal rat. *Vet Hum Toxicol*. 1993; 35:119–122. [PubMed: 8385837]
- Marks AR, Harley K, Bradman A, Kogut K, Barr DB, Johnson C, et al. Organophosphate pesticide exposure and attention in young Mexican-American children: The CHAMACOS study. *Environ Health Persp*. 2010; 118:1768–1774.
- Mazer P, Gischler SJ, MHVDC-VZ, Tibboel D, Bax NM, Ijsselstijn H, et al. Early developmental assessment of children with major non-cardiac congenital anomalies predicts development at the age of 5 years. *Dev Med Child Neurol*. 2010; 52:1154–1159. [PubMed: 21175468]
- Morgan MK, Sheldon LS, Croghan CW, Jones PA, Chuang JC, Wilson NK. An observational study of 127 preschool children at their homes and daycare centers in Ohio: Environmental pathways to cis- and trans-permethrin exposure. *Environ Res*. 2007; 104:266–274. [PubMed: 17258193]
- Morgan MK. Children's exposures to pyrethroid insecticides at home: A review of data collected in published exposure measurement studies conducted in the United States. *Int J Environ Res Public Health*. 2012; 9:2964–2985. [PubMed: 23066409]
- National Center for Health Statistics (NCHS). [accessed July 30 2015] National Health and Nutrition Examination Survey. 2014. Available: http://www.cdc.gov/nchs/nhanes/nhanes_questionnaires.htm
- Olsson AO, Baker SE, Nguyen JV, Romanoff LC, Udunka SO, Walker RD, et al. A liquid chromatography--tandem mass spectrometry multiresidue method for quantification of specific metabolites of organophosphorus pesticides, synthetic pyrethroids, selected herbicides, and deet in human urine. *Anal Chem*. 2004; 76:2453–2461. [PubMed: 15117183]
- Oulhote Y, Bouchard MF. Urinary metabolites of organophosphate and pyrethroid pesticides and behavioral problems in Canadian children. *Environ Health Persp*. 2013; 121:1378–1384.
- Panuwet P, Prapamontol T, Chantara S, Barr DB. Urinary pesticide metabolites in school students from northern Thailand. *Int J Hyg Envir Heal*. 2009; 212:288–297.
- Patrianakos-Hoobler AI, Msall ME, Huo D, Marks JD, Plesha-Troyke S, Schreiber MD. Predicting school readiness from neurodevelopmental assessments at age 2 years after respiratory distress syndrome in infants born preterm. *Dev Med Child Neurol*. 2010; 52:379–385. [PubMed: 20002128]
- Qi X, Zheng M, Wu C, Wang G, Feng C, Zhou Z. Urinary pyrethroid metabolites among pregnant women in an agricultural area of the province of Jiangsu, China. *Int J Hyg Envir Heal*. 2012; 215:487–495.
- Quiros-Alcala L, Mehta S, Eskenazi B. Pyrethroid pesticide exposure and parental report of learning disability and attention deficit/hyperactivity disorder in U.S. Children: NHANES 1999–2002. *Environ Health Persp*. 2014; 122:1336–1342.
- Ratelle M, Cote J, Bouchard M. Toxicokinetics of permethrin biomarkers of exposure in orally exposed volunteers. *Toxicol Lett*. 2015; 232:369–375. [PubMed: 25498136]

- Rodriguez T, van Wendel de Joode B, Lindh CH, Rojas M, Lundberg I, Wesseling C. Assessment of long-term and recent pesticide exposure among rural school children in Nicaragua. *Occup Environ Med.* 2012; 69:119–125. [PubMed: 21725072]
- Santoni G, Cantalamessa F, Spreghini E, Sagretti O, Staffolani M, Piccoli M. Alterations of t cell distribution and functions in prenatally cypermethrin-exposed rats: Possible involvement of catecholamines. *Toxicology.* 1999; 138:175–187. [PubMed: 10593508]
- Tillett T. Optimizing insecticide resources: Global trends in vector control. *Environ Health Persp.* 2012; 120:A164.
- Trunnelle KJ, Bennett DH, Tancredi DJ, Gee SJ, Stoecklin-Marois MT, Hennessy-Burt TE, et al. Pyrethroids in house dust from the homes of farm worker families in the MICASA study. *Environ Int.* 2013; 61:57–63. [PubMed: 24096042]
- Trunnelle KJ, Bennett DH, Tulve NS, Clifton MS, Davis MD, Calafat AM, et al. Urinary pyrethroid and chlorpyrifos metabolite concentrations in northern California families and their relationship to indoor residential insecticide levels, part of the study of use of products and exposure related behavior (SUPERB). *Environ Sci Technol.* 2014; 48:1931–1939. [PubMed: 24422434]
- Viel JF, Warembourg C, Le Maner-Idrissi G, Lacroix A, Limon G, Rouget F, et al. Pyrethroid insecticide exposure and cognitive developmental disabilities in children: The PELAGIE mother-child cohort. *Environ Int.* 2015; 82:69–75. [PubMed: 26057254]
- Wechsler, H. Wechsler Adult Intelligence Scale (WAIS), Spanish version. San Antonio, TX: Psychological Corporation; 1968.
- WHO. Environmental Health Criteria. 1990. Permethrin.
- Young JG, Eskenazi B, Gladstone EA, Bradman A, Pedersen L, Johnson C, et al. Association between in utero organophosphate pesticide exposure and abnormal reflexes in neonates. *Neurotoxicology.* 2005; 26:199–209. [PubMed: 15713341]

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.

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

Table 1

Maternal Characteristics	All			Detects			Non-Detects		
	N	Median	(25th, 75th)	N	Median	(25th, 75th)	N	Median	(25th, 75th)
Age (years)	187	26	(22, 30)	84	28**	(23, 33)	103	24**	(22, 28)
Education (years)	187	11	(9, 12)	84	11*	(9, 12)	103	12*	(9, 12)
IQ Score	187	96	(88, 103)	84	94	(86, 102)	103	97	(91, 105)
SES Score	178	8	(6, 10.5)	77	7*	(6, 10)	101	9*	(7, 10.5)
Blood Lead (µg/dL)	187	5.4	(3.3, 7.8)	84	5.5	(3.4, 8.2)	103	5.3	(3.2, 7.7)
Urinary Specific Gravity	187	1.013	(1.009, 1.017)	84	1.016**	(1.012, 1.019)	103	1.012**	(1.007, 1.014)

* p<0.05,

** p<0.01

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 2

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

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

SD-standard deviation; MDI-Mental Development Index; PDI-Psychomotor Development Index

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

Table 3

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

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;

** NHANES 1999–2002 (NCHS 2014).

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).

	MDI 24				MDI 36			
	N	β	p-value	(95% CI)	N	β	p-value	(95% CI)
All Participants	172				159			
High 3-PBA	37	-3.80	0.11	(-8.44, 0.84)	32	-3.13	0.12	(-7.05, 0.78)
Med 3-PBA	38	-3.54	0.11	(-7.86, 0.78)	36	-0.49	0.78	(-4.04, 3.05)
3-PBA <LOD	97	ref			91	ref		
p for trend			0.07				0.14	
Male	84				77			
High 3-PBA	17	-3.43	0.31	(-10.2, 3.30)	14	-2.28	0.46	(-8.36, 3.80)
Med 3-PBA	15	-2.58	0.43	(-9.10, 3.94)	15	-1.25	0.65	(-6.81, 4.31)
3-PBA <LOD	52	ref			48	ref		
p for trend			0.26				0.43	
Female	88				82			
High 3-PBA	20	-5.54	0.11	(-12.4, 1.32)	18	-3.54	0.21	(-9.08, 1.99)
Med 3-PBA	23	-6.20	0.05	(-12.3, -0.14)	21	-0.13	0.96	(-5.08, 4.83)
3-PBA <LOD	45	ref			43	ref		
p for trend			0.08				0.24	

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).

	PDI 24				PDI 36			
	N	β	p-value	(95% CI)	N	β	p-value	(95% CI)
All Participants	171				159			
High 3-PBA	36	-0.42	0.84	(-4.55, 3.70)	32	-1.55	0.55	(-6.67, 3.58)
Med 3-PBA	38	0.64	0.74	(-3.18, 4.46)	36	-3.08	0.19	(-7.71, 1.56)
3-PBA <LOD	97	ref			91	ref		
p for trend			0.91			0.41		
Male	83				77			
High 3-PBA	16	-0.59	0.85	(-6.72, 5.53)	14	-1.66	0.68	(-9.68, 6.36)
Med 3-PBA	15	2.95	0.32	(-2.93, 8.83)	15	-5.31	0.15	(-12.6, 2.02)
3-PBA <LOD	52	ref			48	ref		
p for trend			0.92				0.44	
Female	88				82			
High 3-PBA	20	-3.32	0.25	(-9.04, 2.40)	18	-0.27	0.94	(-7.45, 6.91)
Med 3-PBA	23	-3.14	0.22	(-8.21, 1.94)	21	-1.00	0.76	(-7.42, 5.42)
3-PBA <LOD	45	ref			43	ref		
p for trend			0.21				0.91	