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Zelalem Adefris Date

Evaluation of Prenatal Pyrethroid Insecticide Exposure, Fetal Growth, and Neurodevelopmental Outcomes in a Thai Agricultural Birth Pilot Cohort

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B.A.
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Abstract

Evaluation of Prenatal Pyrethroid Insecticide Exposure, Fetal Growth, and Neurodevelopmental Outcomes in a Thai Agricultural Birth Pilot Cohort

By Zelalem Negussie Adefris

Recent research suggests that prenatal exposures to pyrethroid insecticides during critical periods of development may impair infant development both physically and neurologically. The present study aimed to assess the relationship between prenatal pyrethroid insecticide exposure, fetal growth outcomes, and neurologic integrity at birth. Neurobehavioral function was measured using seven clusters of the Brazelton Neonatal Behavioral Assessment Scale (BNBAS): Habituation, Orientation, Motor, Range of State, Regulation of State, Autonomic Stability, and Abnormal Reflex. Trimester-resolved concentrations of urinary 3-phenoxybenzoic acid (3-PBA) metabolites of pyrethroid insecticides were measured in order to assess exposures to the fetuses. Our study participants were part of the pilot birth cohort, the **S**tudy of **A**sian **W**omen **A**nd their off**S**pring's **D**evelopment and **E**nvironmental **E**xposures (SAWASDEE), which is comprised of tangerine farmworkers in Northern Thailand and their newborns. Maternal urinary pyrethoid concentrations in the SAWASDEE cohort are lower than concentrations in comparable birth cohorts. In addition, the pyrethroid levels are fairly homogenous with little variation across the samples suggestive of a continual or continuous exposure, most likely from widespread public health applications to control dengue. We observed no statistically significant associations for 3-PBA, fetal growth outcomes, and the BNBAS domains. Total pregnancy ΣDAP and second trimester mercury were significantly associated with orientation performance (β=-0.007, p=0.002 and β=-1.774, p=0.020, respectively). Third trimester ΣDAP was significantly associated with habituation performance (β=-0.002, p=0.009) and second trimester mercury and cadmium were associated with motor performance (β=-1.316, p=0.005 and β=-3.137, p=0.004, respectively). These results suggest that SAWASDEE cohort participants likely have public health pyrethroid exposures that dominate any occupational exposures to pyrethroids. The lower 3-PBA levels combined with higher exposures to other neurotoxicants could have obscured any true associations between pyrethroid exposures and neurobehavioral or fetal growth effects. This study is the first to examine the impact of trimester-specific exposure to pyrethroid insecticides on neurodevelopment and fetal growth at birth.

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I. BACKGROUND & SIGNIFICANCE

Pyrethroid Insecticides

Pyrethroids are present in numerous commercial pesticide formulations and have extensive indoor and outdoor applications, including veterinary, agricultural, public malaria control, and residential usages (Saillenfait et al 2015). Pyrethroid insecticides are considered less toxic to humans than other insecticide classes, such as organophosphates, because they are rapidly metabolized and eliminated from the body (Dewailly et al 2014). Current data from sales and environmental monitoring suggest that, as organophosphate insecticides have been phased out, pyrethroids have become the most popular insecticide for residential pest control (Horton et al 2011). Because of the extensive applications and growing popularity of pyrethroid insecticides, human exposure to these insecticides is likely to increase worldwide. Therefore, it is imperative that the health effects of chronic pyrethroid exposure be defined so that these insecticides may be regulated appropriately.

The acute neurotoxicity of pyrethroid insecticides in human adults is well known (Saillenfait et al 2015). Pyrethroid insecticides act by slowing both the activation and inactivation of voltage-sensitive sodium channels (VSSCs) in insect and mammalian nerves, resulting in disruption of the nervous system (Kolaczinski & Curtis 2004, Shafer et al 2005). Insects do not have the extensive esterase enzyme system that humans do, thus they do not readily metabolize then resulting in acute toxicity to them. Currently, our understanding of the human health effects of chronic pyrethroid exposure on neurodevelopment is both limited and controversial because human and mechanistic studies are few (Saillenfait et al 2015). However, several animal studies have found that exposure to pyrethroids during development has resulted in persistent changes in behavior and neurochemistry (Ahlbom et al 1994, Eriksson & Fresdriksson 1991, Eriksson & Nordberg 1990, Moniz et al 1989, Johri

et al 2006). Another study using human cell lines found that pyrethroids might exhibit harmful effects on nervous system development even at low concentrations (Tran et al 2006).

In the epidemiological literature some studies have found no association between prenatal pyrethroid exposure, fetal growth outcomes, and neurodevelopment (Berkowitz et al 2004, Dabrowski et al 2003, Fiedler et al 2015, Horton et al 2011, Ostrea et al 2012, Viel et al 2015). Conversely, other epidemiological studies have identified links between pyrethroid exposure and ADHD in the general US population, ASD and developmental delay among children whose mothers lived near pyrethroid applications during pregnancy, low birth weight, poorer progress in mental development among those with childhood exposures, and impaired mental progress among prenatally exposed infants (Ding et al, Hanke et al 2003, Horton et al 2011, Shelton et al 2014, Viet et al 2015, Wagner-Schuman et al 2015, Xue et al 2013). A more detailed summary of the epidemiological literature on pyrethroid exposures and children's health can be found in **Table 1.**

Epidemiological studies are necessary to add to the limited but growing evidence of low-level neurotoxicity in children resulting from prenatal pyrethroid insecticide exposure. Furthermore, a lack of information on the specific effects resulting from exposure during critical windows of development limits our understanding of the human health effects of pyrethroids and other insecticides (Quirós-Alcalá et al 2014). The present study will be the first pyrethroid health effect evaluation, in Thailand and elsewhere, to conduct a temporally resolved evaluation of maternal and fetal pyrethroid exposure during each trimester of pregnancy and to assess fetal growth and neurodevelopment after birth.

Prenatal Exposure

Prenatal exposure to pyrethroid insecticides is of particular concern because the fetus is inherently vulnerable to exposures that occur during critical windows of development. We are concerned with prenatal neurodevelopment because the fetus has an immature nervous system and experiences rapid brain growth and development (Viel et al 2015). Pyrethroid compounds create further vulnerability as they can cross the placental barrier and interfere with hormonal, physiological, and neurological development and functionality (Chanda and Pope 1996, Doucet et al 2009, Gupta et al 1985, Muto et al 1992, Johri et al 2006, Miyamoto et al 1995). In addition, some animal studies have found the neurobehavioral changes caused by pyrethroid exposures to be long lasting (Ahlbom et al 1994, Eriksson & Fresdriksson 1991, Eriksson & Nordberg 1990, Moniz et al 1989, Johri et al 2006). Although no human research has confirmed similar effects from developmental exposure to pyrethroids as those seen in animal studies, human case studies have shown that phenytoin, another VSSC inhibitor, is associated with disruptions in nervous system structure and function after developmental exposure (Shafer et al 2005).

Pyrethroid exposures in humans, including pregnant women and infants, have been widely documented (Shafer et al 2005). In Thailand, pesticide use has multiplied four times in the past decade, with pyrethroids and organophosphates as the nation's most widely used insecticides (Panuwet et al 2012). In Thailand, about 40% of women work in agriculture, and many continue their work during pregnancy (Kongtip et al 2013, World Bank 2013). Each year, approximately 200,000 Thai newborns are at risk of prenatal exposure to pesticides due to their mother's occupational exposures associated with agriculture (Lorenz et al 2012). Globally, 43% of the agricultural workforce is comprised of women (United Nations 2014). In South Asian and African countries, the percentage of female agricultural workers nears 70%. Given the rapidly changing demographics of the global agricultural workforce,

understanding the exposure pathways and health effects of pesticide exposures in women, particularly pregnant women, is critical to informing global occupational health and safety efforts.

3-Phenoxybenzoic Acid (3-PBA)

3-PBA is a general chemical metabolite of most commercially available pyrethroids, including cypermethrin, deltamethrin, cyhalothrin, permethrin, and fenvalerate (Saillenfait et al 2015). Thus, detection of 3-PBA in the urine may indicate a number of different environmental sources of exposure to pyrethroid insecticides (Han et al 2008). Studies estimate that the half-life of 3-PBA in urine to be between 6-24 hours after exposure with complete elimination accomplished after several days (Riederer et al 2008).

To establish reference values for comparison 3-PBA metabolite concentrations are assessed in the U.S. general population through the U.S. National Health and Nutrition Examination Survey (NHANES). However, biomonitoring of pyrethroid exposure has only been conducted in Thailand through a few small pilot studies (Thiphom et al 2014, Fiedler et al 2015, Panuwet et al 2008, Panuwet et al 2009). Currently, Thailand has no nationally representative reference value for exposure to pyrethroid insecticides. However, studies to date suggest Thai farmworkers, children, and pregnant women are more highly exposed than the general U.S. population, as may be expected given differences in pesticide regulations (Thiphom et al 2014, Fiedler et al 2015, Panuwet et al 2008, Barr et al 2010, Panuwet et al 2012).

Dialkyl Phosphate Metabolites (DAPs) and Heavy Metals

DAPs are non-toxic metabolites that are widely used to assess exposure to organophosphorus (OP) insecticides (Muñoz-Quezada et al 2013). Approximately 75% of OPs are converted into DAPs, which are detected in urine. DAPs are a sensitive

measurement and can be present in the urine at low-level exposures. A challenge of using DAPs to approximate OP exposure is that they can also be derived from exposure to non-toxic preformed environmental metabolites, as well as a variety of OP pesticides with differing toxicities. However, because our population consists of agricultural workers and we know that the predominate pesticide they use is chlorpyrifos, the likelihood that their DAP levels represent non-toxic pre-formed metabolites is reduced. Analysis of DAPs has been employed in numerous studies of OP exposure in farmers, agricultural workers, pest-control workers, and others (CDC 2013). Studies that have used DAPs analyses in conjunction with BNBAS tests have found significant associations with adverse neurodevelopmental and fetal growth outcomes, including smaller head circumference, abnormal reflexes, attention span, orientation, and motor skills (Young et al 2005, Eskenazi et al 2010, Burns et al 2013, Matthews 2014).

Heavy metals are a group of dense metals and their related compounds. They are usually associated with environmental pollution or toxicity. Heavy metals can be naturally available in the environment or enter the environment through the use of synthetic products, such as pesticides, soldering materials, and paints. Heavy metal exposure can result in a variety of pathways, but for the purpose of the SAWASDEE cohort, we expect the exposure to primarily derive from occupational use of pesticides or from residential sources. Studies have demonstrated that developmental exposure to arsenic, cadmium, mercury, and lead is associated with adverse neurodevelopmental and fetal growth effects (Schenck 2015). These adverse neurodevelopmental and fetal growth effects include birth defects, preterm delivery, miscarriage, stillbirths, low birth weight, infant mortality, lower childhood IQ, decreased exploratory motor activity, and avoidance acquisition (EPA 2007, EPA 2016, Caserta et al 2013). As exposure to heavy metals often co-occur, the associated adverse outcomes could

be from each heavy metal's independent impact or a combined impact. It is important to consider the potential confounding of interactions of numerous toxic substances during prenatal development (Zheng et al. 2014). Consequently, within this analysis of 3-PBA in urine, fetal growth, and neurodevelopmental outcomes we will examine DAPs, arsenic, cadmium, mercury, and lead in our models as potential confounders or effect modifiers.

SAWASDEE Birth Cohort

The **S**tudy of **A**sian **W**omen **a**nd their off**S**pring's **D**evelopment and **E**nvironmental **E**xposures (SAWASDEE[1]) is a birth pilot cohort study in an agricultural region of northern Thailand. This cohort consists of 56 Thai mothers and their children. All of the women enrolled in the SAWASDEE pilot birth cohort work as tangerine farmworkers in Fang District, Chiang Mai Province of Northern Thailand and are occupationally exposed to pyrethroid insecticides. Exposures may also occur through residential and malaria control applications of pyrethroid insecticides. The routes of exposure of primary concern for these women are dermal exposure that occurs from picking or thinning of fruit from trees shortly after they have been sprayed with pesticides and inhalational exposure from re-volatilized pesticide residues. The SAWASDEE cohort improves upon previous studies by measuring pesticide exposure at multiple time points throughout pregnancy, thus capturing monthly and trimester-specific exposures, and increasing its strength and validity in estimating exposures that may be linked to fetal growth and neurodevelopmental outcomes (Matthews 2014).

Brazleton Neonatal Behavioral Assessment Scale

This study will assess the effect of prenatal pyrethroid insecticide exposure, as measured by maternal urinary 3-PBA metabolites, on neurodevelopment at birth as measured by the Brazleton Neonatal Behavioral Assessment Scale (BNBAS). The BNBAS is a standardized

[1] SAWASDEE is a word used for greeting and farewell in Thai and means well-being.

test that assesses neurodevelopment in infants from birth to two months of age (Brazelton & Nugent 2011). The half-hour assessment is comprised of a set of 18 reflexes and 28 behavioral evaluations to assess seven domains of behavior in infants: habituation, orientation, motor, range of state, autonomic stability, and abnormal reflexes (Engel et al 2007, Young et al 2005). Detailed descriptions of the seven domains are in **Appendix 1**. On the six behavioral domains, higher scores represent more optimal functioning. Higher scores represent less optimal function for the reflex cluster. The BNBAS provides insight into the complexity of the neonate, assessing how they handle the transition from an intrauterine, symbiotic condition to a relatively independent experience (Brazelton 1978). In addition, the BNBAS assesses how infants react to internal and external stimuli. It is a universally accepted assessment scale and does not require culturally specific modifications or validation.

II. METHODS

Participants and Recruitment

The Institutional Review Board of Emory University and the Ethic Boards of Chiang Mai University and the Thai Ministry of Health reviewed and approved all study protocols for the SAWASDEE longitudinal birth pilot cohort. The study population consists of female farmworkers and their neonates residing in the Chiang Mai Province of northern Thailand. The women (n=59) were recruited at the local antenatal clinic at Fang Hospital during their first or early second trimester of pregnancy between March 2011 and February 2012.

Inclusion criteria in the SAWASDEE Study are as follows:

1) Aged between 18 and 40 years

2) Thai identification card permitting hospital and antenatal clinic access
3) Thai as the primary language at home
4) Residence in the regional district for at least 6 months and planned residence at least 1 month after delivery
5) Good general health
6) Consumption of fewer than two alcoholic beverages per day and no use of illegal drugs

A Thai identification card allows each pregnant woman a minimum of one prenatal visit to an OB/GYN at Fang Hospital per month. After each participant's initial prenatal visit they were followed longitudinally until three days after delivery. The study had a participation rate of 100%. All women (59/59) who were recruited and that met the inclusion criteria gave their informed consent to be in the study. The retention rate of the study was 95%, due to the fact that three participants were either lost to follow up or excluded because of spontaneous abortion.

Questionnaire and Medical Record Abstraction Data

Participants were administered a questionnaire of 168 questions at the time of their enrollment, at 28 weeks gestation, and at 36 weeks of gestation. The questionnaire collected data on maternal and paternal demographic, housing characteristics, health information, pesticide-use knowledge, attitudes, and practices, among other relevant themes. Medical records were used to obtain additional information, including infant sex, birth weight, birth length, gestational age, head circumference, APGAR scores, and pregnancy or delivery complications.

Exposure Assessment

Maternal urine samples were collected at multiple time points throughout pregnancy, with an average of 8 samples for each woman. Postnatal samples were also collected for some women. Post-natal samples were not included in analysis because, given the short half-lives of pyrethroids and OPs, metabolite levels measured in postnatal urine samples reflect postpartum rather than in-utero exposure. Spot urine samples were collected at the antenatal clinic in a 50 mL polypropylene cup. Each sample was dispensed into smaller vials and stored at -20°C until analysis was conducted at Chiang Mai University in Thailand. Maternal blood samples were collected at the same time points as the questionnaire administration. An additional maternal, infant, and cord blood sample were collected at birth. Two separate maternal and cord blood samples were collected during each time point.

3-PBA metabolites were measured using high-performance liquid chromatography (HPLC) coupled with turbo ion spray atmospheric pressure ionization (TIS) tandem mass spectrometry. A detailed description of the analytical methods and quality control procedures is provided in Olsson et al (2004). For this method, the relative recovery range was 81 - 114%. The relative standard deviations (RSD) for 3-PBA were less than 8%. The reported limit of detection was 0.2 ng/mL of urine for all six common 3-PBA (Olsson et al 2004).

DAP metabolites were measured using gas chromatography (GC) coupled with flame photometric detection (FPD) and internal standard quantification. A detailed description of the analytical methods and quality control procedures is provided in Prapamontol et al. (2013). The relative recovery range for this method was 94.4 - 119% and the relative standard deviations (RSD) were less than 20%. The reported limits of detection ranged from 0.1 ng/mL urine to 2.5 ng/mL urine for all six common DAP metabolites (Prapamontol et al., 2013). For both the 3-PBA and total dialkyl phosphate (ΣDAP) measures, each participant's samples were

averaged across trimesters to create exposure measures for each trimester of pregnancy as well as an overall pregnancy average.

To collect heavy metal measurements, blood samples were collected at enrollment, 28 weeks, and at delivery from the mother, as well as from the umbilical cord at birth. Heavy metals were measured in 4 whole blood samples for each participant. One mL of blood was spiked with yttrium internal standard, mixed, and digested for 3.5 hours with nitric acid. Inductively coupled plasma-mass spectrometry was used to analyze the digested samples for lead, mercury, cadmium, arsenic and chromium. Calibration standards, blanks, and quality control samples were analyzed along with unknown samples. Then, the data was integrated and concentrations were calculated using a linear regression line derived from the standard curve. Lastly, all data were blank-subtracted and quality control samples verified method performance (Schenk 2015).

Neurodevelopmental Outcomes Assessment

Following the BNBAS protocol, a test-certified nurse administered the BNBAS to each infant within three days of birth. Fifty-five infants received the BNBAS, however some infants were missing scores for certain domains. The BNBAS is typically scored using the Lester et al (1982) seven-cluster scoring method. This method divides the assessment's 28 behavioral items and 18 reflexes into seven domains: habituation, orientation, motor performance, range of state, regulation of state, autonomic stability, and abnormal reflexes (Brazelton & Nugent 2011). Each of the behavioral domains is made up of three to six items, which are scored on a nine-point scale. The seventh domain is made up of the 18 reflex items, which are scored on a four-point scale.

For the six behavioral domains, higher scores represent more optimal functioning. Behavioral domain cluster scores are calculated by recoding the original BNBAS items when

necessary. The recoded individual items within each domain are either averaged in order to obtain a summary score. For 15 out of 18 items in the reflex domain, a score of two is considered normal while a score of 0, 1 or 3 is considered abnormal. For the remaining reflex items, scores of 0, 1 or 2 are considered normal while a score of 3 is considered abnormal. The reflex cluster score is a count of the total number of abnormal reflexes exhibited. A higher count indicates less optimal functioning.

Aims & Hypotheses

Aim 1: To assess prenatal exposure to pyrethroid insecticides in the SAWASDEE cohort

Hypothesis 1: Maternal pyrethroid concentrations will vary over the course of pregnancy, indicating the necessity of multiple measures of exposure

Aim 2: To estimate the effect of prenatal exposure to pyrethroid insecticides on neurological integrity at birth as measured using the Brazelton Neonatal Behavioral Assessment Scale (BNBAS)

Hypothesis 2a: Maternal pyrethroid metabolites will be inversely associated with attention parameters as measured by the BNBAS

Hypothesis 2b: Maternal pyrethroid metabolites will be positively associated with abnormal reflexes as measured by the BNBAS

Aim 3: To estimate the effect of prenatal exposure to pyrethroid insecticides on the fetal growth outcomes of gestational age, birth weight, birth length, and head circumference

Hypothesis 3a: Maternal pyrethroid metabolites will be inversely associated with gestational age, birth length, head circumference, and birth weight

Aim 4: To estimate the effects of the combination of prenatal exposures to heavy metals, pyrethroids, and organophosphate insecticides on neurological integrity birth as measured

using the Brazelton Neonatal Behavioral Assessment Scale (BNBAS), as well as fetal growth outcomes

Hypothesis 4a: The combination of prenatal exposures to heavy metals, pyrethroids, and organophosphate insecticides will be inversely associated with attention parameters as measured by the BNBAS

Hypothesis 4b: The combination of prenatal exposures to heavy metals, pyrethroids, and organophosphate insecticides will be positively associated with attention parameters as measured by the BNBAS

Hypothesis 4c: The combination of prenatal exposures to heavy metals, pyrethroids, and organophosphate insecticides will be inversely associated with gestational age, birth weight, birth length, and head circumference

Data Analysis

The data was analyzed using R statistical software, version 3.2.2 (The R Foundation for Statistical Computing, 2015). For Aim I, univariate analysis was conducted to describe exposure and outcomes measures. For Aim II, III, and IV, linear and Poisson regression was utilized. Linear regression was utilized to analyze fetal growth outcomes and six of the seven domains tested in BNBAS. Reflexes were analyzed using Poisson regression, because the data for abnormal reflexes was derived from counts rather than scores.

Based on previous literature, a number of potential confounders and effect modifiers were considered, including gestational age, maternal age, maternal education, infant's sex, and household income. Maternal education and household income were entered as dichotomized variables, as "any" versus "none" for education, or "<6,000 baht per month"

versus ">6,000 baht per month" for income[2], because of the lack of heterogeneity in the multiple categories of each of these variables. Covariates were included in the final models if they caused greater than a 10% change in the coefficient for the exposure predictors when comparing crude estimates to adjusted estimates. Gestational age at delivery and birth weight were not evaluated for confounding because they are potential causal intermediates in the relationship between insecticide exposures, fetal growth, and neurodevelopmental outcomes.

III. RESULTS

Demographic Data

The demographic characteristics of the SAWASDEE pilot birth cohort participants can be found in **Table 2**. The majority of the participants were young women (mean age = 26 years) born in Burma (64.3%) and of the Thai Yai ethnicity (60.7%). The majority of participants had low educational attainment (64.3% had never attended school). Most participants (66.1%) also had household incomes below the Thai poverty line, living on 6,000 Baht ($200) or less per month.

The majority of participants enrolled in the study during their second trimester of pregnancy (71.4%) as compared to the 28.6% that enrolled in their first trimester of pregnancy. The season of year with the greatest enrollment was the rainy season, May-October, when pesticides are not actively applied. The SAWASDEE cohort included eight infants who were preterm (born before 37 weeks gestation) and seven infants who were low birth weight (weighing less than 2500 g), including four low birth weight and preterm infants.

[2] 6000 baht ~ 200 USD. Incomes below 6000 baht/month are considered below the poverty level.

Exposure Distributions

Exposure distributions for DAP metabolite concentrations over the course of pregnancy are shown in **Table 3**. Eight biological samples were collected on average from each participant, ranging from 5 to 13 samples. The mean and median urinary metabolite levels for the average of all pregnancy measurements of total the DAPs were 366.9 nmol/L and 208.9 nmol/L, respectively. Urinary metabolite levels measured during the first trimester are higher compared to second trimester levels, which are higher compared to third trimester levels. First trimester urinary metabolite levels showed the most variation compared to second and third trimester levels.

Exposure distributions for pyrethroid metabolite concentrations over the course of pregnancy are shown in **Table 3**. Eight biological samples were collected on average from each participant, ranging from 5 to 13 samples. The mean and median urinary metabolite levels for the average of all pregnancy measurements of 3-PBA were 0.5 ng/mL and 0.3 ng/mL, respectively. Urinary metabolite levels stayed consistent over the course of all three trimesters. Second trimester urinary metabolite levels showed the most variation compared to first and third trimester levels.

Exposure distributions for heavy metal concentrations during the pregnancy are presented in **Table 3**. The amount of samples at the second and third timepoints varied due to a variety of factors, including being unable to obtain a blood sample, refusal, hydration status, and other medical reasons. The mean level of arsenic was greatest at the third trimester (2.1 ng/mL) and the lowest in the first trimester (1.5 ng/mL). For cadmium, the mean level was greatest was at the third trimester (0.7 ng/mL) and lowest at the second trimester (0.2 ng/mL). For mercury, the average concentration was greatest at the second trimester (1.9 ng/mL) but was the lowest during the third trimester (1.3 ng/mL). The mean

lead level was highest during the third trimester (27.9 ng/mL) and lowest at the second trimester (21.4 ng/mL).

Outcome Distributions

The sample means, medians, standard deviations, and ranges for the summed measures of each of the seven cluster scores that comprise BNBAS are presented in **Table 4**. These cluster scores were used in the linear and negative binomial regression models. The cluster with the greatest range was the Orientation cluster, with a standard deviation of 7.3 and range between 14.0-51.0. In contrast, the cluster with the least variation was Regulation of State (range 15.0-17.5). Excluding the Abnormal Reflex cluster, the infants in this study performed best on the Habituation cluster as demonstrated by their high mean (26.5) and median (27.0) scores when compared to the highest possible cluster score (36). Autonomic Stability was the cluster with the lowest mean (10.5). Regarding the Abnormal Refluxes cluster, the average infant had approximately one abnormal reflex. The range for this cluster is 0.0 to 6.0, with more abnormal reflexes indicating less optimal performance.

Linear Regression Analysis

A multiple linear regression was conducted to see exposure to 3-PBA, heavy metals, and ΣDAP predicted BNBAS domain performance, excluding abnormal reflexes, as well as the fetal growth outcomes of gestational age, head circumference, birth weight, and birth length. The initial models underwent backwards, forwards, and stepwise model selection methods. The results of the statistically significant linear regression models for six BNBAS clusters, excluding abnormal reflexes, for total pregnancy, first trimester, second trimester, and third trimester exposures are presented in **Tables 5-16**.

It was found that total pregnancy ΣDAP and mercury exposures explain a significant amount of the variance in the BNBAS Orientation domain (Adjusted R^2=0.25), while

controlling for maternal age and urinary creatinine levels. A significant association with increased total preganancy ΣDAP and Orientation was observed (β=-0.007, p=0.002), indicating less optimal performance on the Orientation cluster with increasing total pregnancy ΣDAP metabolite levels (**Table 5**).

For first trimester exposures, arsenic exposures explain a significant amount of the variance in the BNBAS Orientation domain (Adjusted R^2=0.35, β=-4.327, p=0.007) indicating less optimal performance on the Orientation domain with increasing arsenic exposure (**Table 6**). Arsenic, ΣDAP, mercury, and cadmium explain a significant amount of the variance in infant birth weight (Adjusted $R^{2=}$0.75) while controlling for maternal education, maternal age, and urinary creatinine levels. The significant associations of arsenic, mercury, and cadmium (β=-753.938, p=0.0003, β=-156.177, p=0.036, β=-671.138, p=0.008, respectively) indicate a decrease in infant birth weight with increasing exposure levels. The significant association of ΣDAP (β=0.257, p=0.008) indicates an increase in infant birth weight with increasing exposure levels (**Table 7**). Lastly, cadmium and lead explain a significant amount of the variance in infant head circumference (Adjusted R^2=0.44), while controlling for maternal age, maternal education, and household income. These findings indicate smaller head circumferences with increasing cadmium exposures (β=-2.736, p=0.029) and larger head circumferences with increasing lead exposures (β=0.091, p=0.006) (**Table 8**).

For second trimester exposures, ΣDAP, mercury, and cadmium exposures explain a significant amount of the variance in the BNBAS Orientation domain (Adjusted R^2=0.29), while controlling for urinary creatinine levels. The significant association of mercury (β=-1.774, p=0.020) indicates less optimal performance on the Orientation domain with

increasing exposure levels (**Table 9**). While adjusting for household income, cadmium exposures explain a significant amount of the variance in the BNBAS Range of State domain (Adjusted R^2=0.19, β=3.040, p=0.010), indicating more optimal performance on the Range of State domain with increasing cadmium exposure (**Table 10**). ΣDAP, lead, and cadmium explain a significant amount of the variance in gestational age (Adjusted $R^{2=}$0.28) while controlling for household income, maternal age and urinary creatinine levels. The significant association of lead (β=0.043, p=0.031) indicates an increase in gestational age with increasing exposure levels. The significant association of ΣDAP (β=-0.001, p=0.019) indicates a decrease in gestational age with increasing exposure levels (**Table 11**).

For third trimester exposures ΣDAP, arsenic, and lead exposures explain a significant amount of the variance in the BNBAS Habituation domain (Adjusted R^2=0.14), while controlling for maternal education and urinary creatinine levels. The significant association of ΣDAP (β=-0.002, p=0.009) indicates less optimal performance on the Habituation domain with increasing exposure levels (**Table 12**). Mercury and lead explain a significant amount of the variance in the Orientation domain (Adjusted $R^{2=}$0.18). The significant association of lead (β=-0.195, p=0.002) indicates less optimal performance on the Orientation domain with increasing exposure levels (**Table 13**). Arsenic, cadmium, mercury, and lead explain a significant amount of the variance in the Motor domain (Adjusted $R^{2=}$0.31), while adjusting for maternal age. The significant associations of mercury, cadmium, and lead (β=-1.316, p=0.005, β=-3.137, p=0.004, and β=-0.065, p=0.041, respectively) indicate less optimal performance on the Motor domain with increasing exposure levels (**Table 14**). Arsenic and mercury explain a significant amount of the variance in the Autonomic Stability domain (Adjusted $R^{2=}$0.21) while controlling for household income. The

significant associations of mercury and arsenic (β=0.447, p=0.047 and β=0.217, p=0.014, respectively) indicate more optimal performance on the Autonomic Stability domain with increasing exposure levels (**Table 15**). Lead explains a significant amount of the variance in the Range of State domain (Adjusted R^2=0.06, β=-0.057, and p=0.045) its significant association indicates less optimal performance on the Range of State domain with increasing exposure levels (**Table 16**).

Poisson Regression Analysis

Negative binomial and Poisson regression models were conducted to see if exposures to 3-PBA, heavy metals, and ΣDAP predicted BNBAS Abnormal Reflexes domain performance. No models resulted in statistically significant associations after controlling for urinary creatinine (**Table 17**).

IV. DISCUSSION

Interpretation of Results

Compared to other studies of pyrethroid exposure in pregnant women in China and the Caribbean, the SAWASDEE cohort participants have much lower levels of 3-PBA (Xue et al 2013, Ding et al 2015, Berkowitz et al 2004, Dewailly et al 2014) as demonstrated in **Figure 1**. In addition, the interquartile range of 3-PBA among low-income pregnant women in New York City was 2.4-69.8 ng/mL, whereas the interquartile range in our study population is 0.19-0.41 ng/mL (Berkowitz et al). This difference is likely due to the widespread indoor use of pyrethroid insecticides for pest control efforts in New York City. This is a contrast to the exposure distributions of the ΣDAP metabolites in the SAWASDEE cohort, as they are much higher than those reported in the prospective cohort studies conducted in Young et al (2005) and Engel et al (2007) (Matthews 2014). This, along with the low variation in median

3-PBA levels in different trimesters of pregnancy indicates that the SAWASDEE population has greater occupational exposures to organophosphate pesticides over pyrethroid pesticides, and that pyrethroid exposure may be primarily through malaria-control. Residential applications of pyrethroids may contribute to the low urinary metabolite levels, to some extent, but their use in homes is fairly rare in this high poverty region. Furthermore, these low-level pyrethroid exposures could have obscured any real associations with neurobehavioral or fetal growth effects.

The results of the SAWASDEE pilot birth cohort are suggestive of adverse neurodevelopmental effects from prenatal organophosphate pesticide and heavy metal exposures as measured by ΣDAP metabolites, as well as maternal blood levels of arsenic, lead, mercury, and cadmium. Although the study found statistically significant associations for the fetal growth outcomes of birth weight, head circumference, and gestational age, these results should be interpreted cautiously because of their small sample sizes and their high likelihood of being over-fit, statistically. For these same reasons, the statistically significant findings of an inverse relationship between the Orientation domain and mercury levels should also be interpreted with caution.

Our other findings include an association between total pregnancy ΣDAP metabolites and sub-optimal performance on the BNBAS Orientation domain. In addition, we found a positive association between second trimester cadmium levels and performance on the Range of State domain. For third trimester measures we found statistically significant associations between sub-optimal performance on the Habituation domain and ΣDAP metabolites, the Orientation and Range of State domains and lead, and the Motor domain and mercury, cadmium, and lead. In addition, an association between more optimal performance on the Autonomic Stability domain and arsenic and mercury levels was seen.

Lastly, we observed no associations between the abnormal reflexes domain and our populations' pesticide and heavy metal exposures.

To date, a handful of studies have utilized a prospective birth cohort design to evaluate the impacts of pyrethroid exposures on neurodevelopment and fetal growth (**Table 1**). Berkowitz et al (2004), Ding et al (2015), and Xue et al (2013) evaluated the relationship between fetal growth measures, maternal PBA, and PON-1 activity using a prospective cohort study in New York City, NY, USA. They found no association between PBA metabolite levels and fetal growth outcomes. Berkowitz et al collected only a single sample of maternal blood during the third trimester to assess pregnancy PBA exposures. Ding et al also evaluated the relationship between fetal growth and pyrethroid exposures through a birth cohort in rural northern China. Their metabolites of interest were cis-DCCA, trans-DCCA, and 3-PBA, which were collected during a single maternal urine sample a few days before birth. They found an inverse relationship between birth weight and the sum of cis-DCCA, trans-DCCA, and 3-PBA exposures taken together.

Ostrea et al evaluated the relationship between pyrethroid exposures and neurodevelopmental outcomes via a birth cohort in an agro-industrial province of the Philippines. The pyrethroid metabolites of bioallethrin, cypermethrin, cyfluthrin, and transfluthrin were gathered from maternal blood and hair at mid-gestation, as well as through cord blood, infant meconium, and infant hair after birth. They found no association between results of the Griffith Mental Test and measures of pyrethroid exposure. Horton et al (2011) studied a New York City birth cohort to evaluate the relationship between pyrethroid exposures and 36-month neurodevelopment. They measured permethrin and piperonyl butoxide in cord plasma at delivery and personal air samples during the third trimester of pregnancy. Horton et al found an inverse relationship between increased piperonyl butoxide

exposures as measured by personal air samples and more optimal functioning on the Mental Development Index. Viel et al studied a French birth cohort to assess the relationship between prenatal and childhood pyrethroid exposures and cognitive developmental disabilities at six years of age. The prenatal pyrethroid metabolites of 3-PBA, 4-F-3-PBA, cis-DCCA, cis-DBCA, and trans-DCCA were gathered from a single maternal urine sample in the first or second trimester of pregnancy. Viel et al found no association between prenatal pyrethroid metabolite concentrations and childhood cognitive scores at age six. Lastly, a birth cohort study in China's Henan province measured infant growth outcomes as well as neurodevelopment through the Development Screen Test at 1 year of age. They collected a single maternal urine sample at birth and found that synthetic pyrethroid pesticide exposure was negatively related to the neurodevelopment of infants.

All of the aforementioned studies relied upon samples taken at only one or two points in pregnancy to characterize prenatal exposure for the duration of pregnancy. In addition, all but two of the studies relied only on samples taken during the third trimester and only half of the studies relied on samples taken more than 4 days before birth. It is preferable to rely on more biological samples taken over the course of pregnancy because otherwise samples will be less representative of long-term exposure than regular measurements (Bouchard et al 2011, Morgan et al 2016). In addition, these studies have been unable to assess trimester specific-exposures, particularly those in early pregnancy, on fetal growth and infant neurodevelopment. The present study collected an average of 8 maternal urine samples per participant. As a result, we can more accurately characterize prenatal heavy metal and pesticide exposures than previous studies. In addition, we have been able to evaluate the effect of trimester specific exposures on fetal growth outcomes and neurodevelopment. Given that the neuronal pathways that control orientation and refined motor skills are mostly developed during the

second trimester and that neural migration and spinal cord formation that controls more primitive functions such as reflex are believed to be developed during the late 1st to early 2nd trimester, it is important to assess if exposures cause perturbations or insults during the formation of these pathways that can adversely affect their functioning after birth (Rice and Barone, 2000).

Limitations

The half-lives of pyrethroid insecticides are short, ranging from 6 to 24 hours. Given these short half-lives our measurements may not capture the full range of exposures that occurred over the course of pregnancy, despite the fact that we took several samples per participant over the course of pregnancy. However, given the relatively low variation in levels likely from continual/continuous exposures, this potential limitation was probably not a problem in our study. Although we attempted to characterize first trimester exposures, we only obtained twenty urine samples and seventeen blood samples during the first trimester of pregnancy. Due to this small sample size and the risk that a number of our first and second trimester regression models are at risk of being over-fit, our ability to accurately investigate associations between exposures and fetal growth or BNBAS outcomes was limited.

The metabolite 3-PBA is a general metabolite for most pyrethroid pesticides. However, utilizing additional pyrethroid metabolites would allow us to identify exposures to different chemicals within the pyrethroid chemical class, which could help to identify different sources of exposure. Previous researchers have found correlations between neurobehavioral outcomes and urinary pyrethroid metabolites other than 3-PBA and the sum of several pyrethroid metabolites including 3-PBA (Xue et al, Ding et al). This study did not have the opportunity to explore these potential associations

The ability of the BNBAS when administered at birth to predict life-long neurological development is largely unknown. In addition, it is possible that outcome misclassifications may have occurred, as only one BNBAS was administered to each infant. Any potential outcome misclassifications are likely non-differential with respect to exposure, resulting in a bias toward the null.

Although we considered a number of confounders during this analysis, including maternal education, household income, maternal age, and infant's sex, there are additional confounders we were unable to assess. These confounders include paternal IQ and exposure to other known neurotoxicants, such as tobacco smoke, alcohol, and organochlorine pesticides. Our population was largely homogenous in terms of maternal education and household income; however, some residual confounding could be present.

Despite these limitations, this study is progressive. It is the first study to examine trimester-specific exposures to pyrethroid pesticides on fetal growth and infant neurodevelopment. Further investigation is needed to confirm these results.

V. CONCLUSIONS & RECOMMENDATIONS

Summary

Our results indicate that infants in the SAWASDEE pilot birth cohort experience low, likely continual, prenatal exposures to pyrethroid pesticides, high exposures to organophosphate pesticides, and high exposures to heavy metals when compared to other birth cohorts around the world. As a result of the organophosphate and heavy metal exposures in particular, more highly exposed infants performed less optimally on an assessment of neurologic integrity at birth and exhibited some measures of impaired fetal growth. This study suggests no association between low pyrethroid exposures, neurologic

integrity, and impaired fetal growth. It is also suggestive of an adverse association between heavy metal and organophosphate exposures, fetal growth, and neurologic integrity at birth. This study demonstrates a great need for additional studies that utilize temporally resolved prenatal exposure data to further investigate these associations.

Recommendations for Future Research

Further research is needed in order to understand how pyrethroid exposures influence fetal growth and infant neurodevelopment. Previous research has not investigated trimester-specific exposures due to a limited number of biological samples collected throughout pregnancy. The present study was unable to fully investigate the potential consequences of trimester-specific exposures due to a small sample size of first and second pregnancy trimester measurements. Additionally, the present study was unable to fully investigate specific pyrethroid exposures as it only investigated a single general pyrethroid metabolite, 3-PBA. Future studies should be designed to recruit participants pre-pregnancy, incorporate a high number of biomarker measurements throughout pregnancy, and measure other pyrethroid metabolites, such as trans- and cis-DCCA, in order to better characterize exposures over the course of pregnancy.

To determine the accuracy of the BNBAS, further research is necessary to determine if the neurodevelopmental patterns identified at birth continue throughout childhood. This is important in determining if BNBAS are accurate predictive measurements of neurodevelopmental outcomes throughout life. The SAWASDEE longitudinal birth cohort, as well as future studies, should follow children as they age to assess the accuracy of BNBAS scores in predicting neurodevelopmental outcomes.

Policy Recommendations

Prenatal exposure to neurotoxic pesticides and heavy metals is a public health risk for populations across the world. The risk of prenatal pesticide exposures increases in developing nations, such as Thailand, where pesticide regulations are absent or unenforced. There are a number of studies that have evaluated the relationship between pyrethroid pesticides and adverse health outcomes, including neurodevelopment and fetal growth, in diverse populations across the world (Ding et al, Hanke et al 2003, Horton et al 2011, Shelton et al 2014, Viet et al 2015, Wagner-Schuman et al 2015, Xue et al 2013). Studies have also found relationships between adverse neonatal outcomes and prenatal exposures to organophosphate pesticides and heavy metals (Bouchard et al 2011, Engel et al 2011, Eskenazi et al 2007, Marks et al 2010, Munoz-Quezada et al 2013, Rauh et al 2006, Rauh et al 2012). However, the absence of trimester-specific exposure data contributed to the prevention of translating this research into policy. The SAWASDEE birth cohort contributes to this field of research by incorporating time-resolved exposure data. The ultimate goal of this study, along with the future planned studies in Thailand, is to better protect maternal and child health by informing regulatory reform that will decrease harmful pesticide and heavy metal exposures at critical periods of fetal growth.

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VII. TABLES AND FIGURES

Table 1. Summary of Epidemiological Literature Review of Prenatal & Early-Life Pyrethroid Exposures, Fetal Growth Outcomes, and Neurodevelopment

Author, Year	Biomarker(s)	Neurobehavioral Outcome(s) or Test(s)	Fetal Growth Outcome(s)	Findings
Berkowitz et al, 2004	3-PBA and PON-1	n/a	Birth Weight, Birth Length, Gestational Age, Head Circumference	No association
Dabrowski et al, 2003	n/a (Reported pesticide use)	n/a	Birth Weight	No association
Ding et al, 2015	cis- and trans-DCCA, 3-PBA	n/a	Birth Weight, Birth Length, Gestational Age, Head Circumference	Inverse relationship between pyrethroid exposure and birth weight
Fiedler et al, 2015*	cis- and trans-DCCA, 3-PBA	Behavioral Assessment and Research System Test	n/a	No association
Hanke et al, 2003	n/a (Reported pesticide use)	n/a	Birth Weight	Inverse relationship between first and second-trimester reported pyrethroid exposure and birth weight
Horton et al, 2011	cis- and trans-permethrin	Bayley Scales of Infant Development II with Mental and Pyschomotor Development Indices	n/a	Higher exposures to piperonyl butoxide in personal air samples scored lower on the Mental Development Index than those with lower exposures
Ostrea et al, 2012	bioallethrin, cypermethrin, cyfluthrin, transfluthrin	Griffith Mental Test	n/a	No association
Shelton et al, 2014	n/a (Spatial Model)	Autism Diagnostic Interview - Revised, Autism Diagnostic Observation Schedule, Social Communications Questionnaire, Mullen Scales of Early Learning, Vineland Adaptive Behavioral Scale	n/a	Children of mothers residing near pyrethroid applications just before conception or during third trimester were at greater risk for autism spectrum disorders and developmental delay
Viel et al, 2015*	3-PBA, 4-F-3-PBA, cis-DCCA, cis-DBCA, trans-DCCA	Wechsler Intelligence Scale for Children Verbal Comprehension Index, Wechsler Intelligence Scale for Children Working Memory Index	n/a	Childhood 3-PBA and cis-DBCA concentrations negatively associated with verbal comprehension and working memory scores. No associations between maternal prenatal pyrethroid metabolite concentrations and child cognitive scores.
Wagner-Schuman et al, 2015*	3-PBA	ADHD Diagnostic Interview for Children	n/a	Children with urinary 3-PBA above the limit of detection (LOD) were twice as likely to have ADHD as

				those below the LOD, and positive relationship between hyperactive-impulse symptoms and urinary 3-PBA
Xue et al, 2013	cis- and trans-DCCA, 3-PBA	Development Screen Test	Height, weight, head, and chest circumference at 1 year old	Inverse relationship between pyrethroid exposure and infant neurodevelopment

*Researched at childhood exposures

Table 2. Demographic characteristics of the SAWASDEE pilot birth cohort, Chiang Mai Province, Thailand, 2011-2012 (n = 56)

	n	**%**
Marital Status		
Married	5	8.9%
Living as Married	51	91.1%
Marital Ethnicity		
Thai	11	19.6%
Thai Yai	34	60.7%
Chinese	2	3.6%
Other	9	16.1%
Maternal Country of Birth		
Thailand	19	33.9%
Burma	36	64.3%
China	1	1.8%
Maternal Education		
None, never attended school	36	64.3%
Primary School	10	17.9%
Junior High/High School	2	3.6%
High School, No Diploma	7	12.5%
Attended some college	1	1.8%
Household Income		
1,5000 Baht or less*	1	1.8%
1,501 to 3,000 Baht*	9	16.1%
3,001 to 6,000 Baht*	27	48.2%
6,001 to 9,000 Baht	10	17.9%
9,001 to 12,000 Baht	3	5.4%
More than 12,000 Baht	3	5.4%
Trimester of Enrollment		
First (0 – 12 weeks)	16	28.6%
Second (12 – 24 weeks)	40	71.4%
Season of Enrollment		
Dry (November – January)	11	19.6%
Hot (February - April)	11	19.6%
Rainy (May – October)	34	60.7%
Preterm Birth (≤37 weeks)		
Yes	8	14.3%
No	48	85.7%
Low Birth Weight (≤2,500 grams)		
Yes	7	12.5%
No	49	87.5%
Infant Sex		
Male	28	50.0%
Female	28	50.0%

Maternal Pre-pregnancy BMI		
Underweight	7	12.7%
Normal	44	80.0%
Overweight	3	5.5%
Obese	1	1.8%

*6,000 Baht ~ 184.15 USD; Incomes below 6,000 baht/month are considered below the poverty level

	n	*Mean*	*SD*	*Range*
Maternal Age	56	26.3	4.7	18.0-35.0
Maternal Pre-Pregnancy BMI	56	20.9	2.6	16.4-30.2
Birth Weight (in grams)	56	2826.5	420.3	1560.0-3750.0
Samples Collected During Pregnancy	56	8.0	1.8	5.0-13.0
Gestational Age at Enrollment	56	14.8	3.1	8.0-23.0
Gestational Age at Delivery	56	38.6	1.3	35.0-41.0
Head Circumference	56	32.8	1.7	28.0-37.0
Birth Length	56	51.5	2.7	41.0-56.0

Table 3. Prenatal Heavy Metal Levels (ng/mL), Prenatal Dialkylphosphate Metabolite Levels (nmol/L), & Prenatal 3-Phenoxybenzoic Acid Levels (ng/mL)

Metabolite of Exposure	*n*	*Mean (ng/mL)*	*Median (ng/mL)*	*SD (ng/mL)*	*Range (ng/mL)*	*IQR (ng/mL)*
3-PBA						
Total Pregnancy	56	0.5	0.3	0.4	0.2 – 3.1	0.3
First Trimester	20	0.8	0.3	1.7	0.2 – 7.4	0.3
Second Trimester	55	0.5	0.3	1.0	0.2 – 7.8	0.2
Third Trimester	56	0.4	0.3	0.4	0.2 – 2.0	0.3
Arsenic						
Total Pregnancy	56	1.9	1.4	1.4	0.8 – 10.3	0.7
First Trimester	17	1.5	1.3	0.9	0.6 – 4.6	0.7
Second Trimester	33	1.7	1.3	1.7	0.3 – 10.2	1.1
Third Trimester	55	2.1	1.6	2.4	0.9 – 18.5	0.5
Cadmium						
Total Pregnancy	56	0.5	0.4	0.3	0.0 – 1.2	0.5
First Trimester	17	0.3	0.1	0.4	0.0 – 1.5	0.6
Second Trimester	33	0.2	0.1	0.4	0.0 - 1.8	0.4
Third Trimester	55	0.7	0.7	0.4	0.0 – 2.3	0.3
Mercury						
Total Pregnancy	56	1.5	1.4	0.9	0.2 – 5.1	1.2
First Trimester	17	1.7	1.4	1.2	0.2 – 4.1	1.2
Second Trimester	33	1.9	1.5	1.5	0.0 – 8.0	1.5
Third Trimester	55	1.3	1.1	0.9	0.1 - 4.8	1.1
Lead						
Total Pregnancy	56	25.5	23.0	12.1	2.9 – 65.4	9.7
First Trimester	17	26.3	19.4	19.0	2.86 – 70.6	17.6
Second Trimester	33	21.4	22.2	9.3	7.2 – 53.0	11.9
Third Trimester	55	27.9	23.5	13.4	2.9 – 66.8	11.0

Metabolite of Exposure	*n*	*Mean (nmol/L)*	*Median (nmol/L)*	*SD (nmol/L)*	*Range (nmol/L)*	*IQR (nmol/L)*
DAPs						
Total Pregnancy	56	366.9	208.9	384.7	42.4 – 1759.0	395.0
First Trimester	19	804.4	133.3	1586.2	27.5 – 6432.0	635.4
Second Trimester	55	405.0	199.2	508.2	27.5 – 2898.0	405.0
Third Trimester	56	301.8	124.2	341.2	26.7 – 1393.0	450.0

Table 4. BNBAS Summed Cluster Scores for Study Sample

	n	*Mean*	*Median*	*SD*	*Range*	*IQR*	*Highest Possible Score*
Habituation	55	26.5	27.0	2.7	13.0 – 31.0	25.0 – 28.0	36
Orientation	55	38.0	39.0	7.3	14.0 – 51.0	34.0 – 43.5	63
Motor	55	25.8	27.0	4.5	11.0 – 35.0	24.0 – 28.0	45
Range of State	55	22.2	22.0	2.7	16.0 – 27.0	20.0 – 24.0	36
Regulation of State	55	16.4	16.0	2.7	13.0 – 26.0	15.0 – 17.5	36
Autonomic Stability	54	10.5	10.5	1.6	9.0 – 11.0	9.0 – 15.0	27
Abnormal Reflexes	54	1.1	1.0	1.2	0.0 – 6.0	0.0 – 1.0	n/a

Table 5. Coefficients for statistically significant multiple linear regression models of total pregnancy average prenatal 3-phenoxybenzoic acid, dialkylphosphate metabolite, and heavy metal analyte levels

Table 5.
Outcome: BNBAS Orientation Domain[a]
N: 55 Adjusted R2: 0.25 P-value: .001

Coefficients	Beta Estimate	Standard Error	t-statistic	p-value
Intercept*	37.902	6.058	6.256	<0.0001
DAP*	-0.007	0.002	-3.258	0.002
Mercury	-1.529	0.939	-1.628	0.110

[a] Adjusted for maternal age and urinary creatinine
* Statistically significant

Table 6-8. Coefficients for statistically significant multiple linear regression models of first trimester average prenatal 3-phenoxybenzoic acid, dialkylphosphate metabolite, and heavy metal analyte levels

Table 6.
Outcome: BNBAS Orientation Domain
N: 17 Adjusted R2: 0.35 P-value: .007

Coefficients	Beta Estimate	Standard Error	t-statistic	p-value
Intercept*	20.828	2.438	8.541	<0.0001
Arsenic*	-4.327	1.382	-3.131	0.007

* Statistically significant

Table 7.
Outcome: Infant Birth Weight[a]
N: 17 Adjusted R2: 0.75 P-value: .003

Coefficients	Beta Estimate	Standard Error	t-statistic	p-value
Intercept*	2641.795	432.536	6.108	0.0002
DAP*	0.257	0.075	3.423	0.008
Arsenic*	-753.938	131.563	-5.731	0.0003

Mercury*	-156.177	63.397	-2.463	0.036
Cadmium*	-671.138	207.215	-3.239	0.008

[a] Adjusted for maternal education, maternal age, and urinary creatinine
* Statistically significant

Table 8.
Outcome: Infant Head Circumference[a]
N: 16 Adjusted R2: 0.44 P-value: .048

Coefficients	Beta Estimate	Standard Error	t-statistic	p-value
Intercept*	28.843	2.330	12.379	<0.0001
Cadmium*	-2.736	1.071	-2.556	0.029
Lead*	0.091	0.026	3.503	0.006

[a] Adjusted for maternal education, maternal age, and household income
* Statistically significant

Table 9-11. Coefficients for statistically significant multiple linear regression models of second trimester average prenatal 3-phenoxybenzoic acid, dialkylphosphate metabolite, and heavy metal analyte levels

Table 9.
Outcome: BNBAS Orientation Domain[a]
N: 31 Adjusted R2: 0.29 P-value: .011

Coefficients	Beta Estimate	Standard Error	t-statistic	p-value
Intercept*	19.808	3.657	5.416	<0.0001
DAP	-0.004	0.002	-1.946	0.063
Mercury*	-1.774	0.714	-2.483	0.020
Cadmium	4.166	2.678	1.556	0.132

[a] Adjusted for urinary creatinine
* Statistically significant

Table 10.
Outcome: Range of State Domain[a]
N: 28 Adjusted R2: 0.19 P-value: .027

Coefficients	Beta Estimate	Standard Error	t-statistic	p-value
Intercept*	5.900	0.732	8.059	<0.0001
Cadmium*	3.040	1.085	2.802	0.010

[a] Adjusted for household income
* Statistically significant

Table 11.
Outcome: Gestational Age Domain[a]
N: 29 Adjusted R2: 0.28 P-value: .039

Coefficients	Beta Estimate	Standard Error	t-statistic	p-value
Intercept*	37.288	1.260	29.599	<0.0001
DAP*	-0.001	0.000	-2.506	0.019
Lead*	0.043	0.019	2.277	0.031
Mercury	0.168	0.109	1.530	0.138

[a] Adjusted for maternal age and urinary creatinine
* Statistically significant

Table 12-16. Coefficients for statistically significant multiple linear regression models of third trimester average prenatal 3-phenoxybenzoic acid, dialkylphosphate metabolite, and heavy metal analyte levels

Table 12.
Outcome: BNBAS Habituation Domain[a]
N: 54 Adjusted R2: 0.14 P-value: .032

Coefficients	Beta Estimate	Standard Error	t-statistic	p-value
Intercept*	8.819	0.973	9.063	<0.0001
DAP*	-0.002	0.001	-2.716	0.009
Arsenic	-0.125	0.120	-1.048	0.300
Lead	0.036	0.022	1.660	0.103

[a] Adjusted for maternal education and urinary creatinine
* Statistically significant

Table 13.
Outcome: BNBAS Orientation Domain
N: 54 Adjusted R2: 0.18 P-value: .002

Coefficients	Beta Estimate	Standard Error	t-statistic	p-value
Intercept*	21.938	2.221	9.878	<0.0001
Mercury	-1.593	0.878	-1.813	0.076
Lead*	-0.195	0.059	-3.280	0.002

* Statistically significant

Table 14.
Outcome: BNBAS Motor Domain[a]
N: 53 Adjusted R2: 0.31 P-value: .0003

Coefficients	Beta Estimate	Standard Error	t-statistic	p-value
Intercept*	12.343	2.630	4.693	<0.0001
Arsenic	-0.301	0.174	-1.728	0.091
Mercury*	-1.316	0.450	-2.924	0.005

Cadmium*	-3.137	1.044	-3.005	0.004
Lead*	-0.065	0.031	-2.106	0.041

[a] Adjusted for maternal age
* Statistically significant

Table 15.
Outcome: BNBAS Autonomic Stability Domain[a]
N: 50 Adjusted R2: 0.21 P-value: .003

Coefficients	Beta Estimate	Standard Error	t-statistic	p-value
Intercept*	1.181	0.436	2.707	0.009
Arsenic *	0.217	0.085	2.543	0.014
Mercury*	0.447	0.220	2.036	0.047

[a] Adjusted for household income
* Statistically significant

Table 16.
Outcome: BNBAS Range of State Domain[a]
N: 52 Adjusted R2: 0.06 P-value: .045

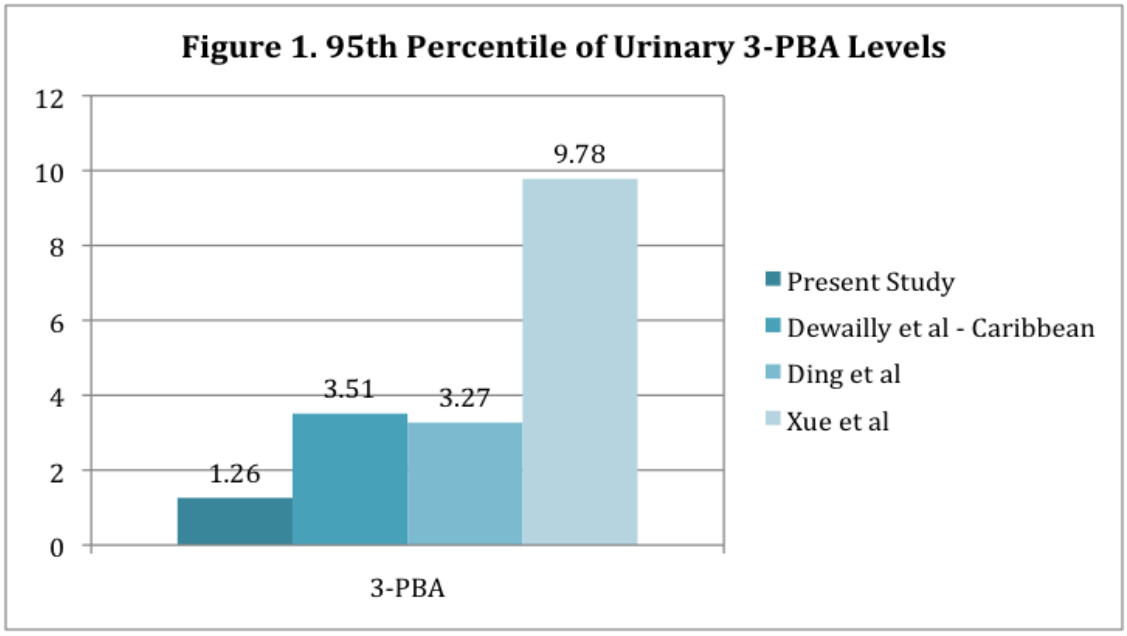
Coefficients	Beta Estimate	Standard Error	t-statistic	p-value
Intercept*	8.762	0.838	10.460	<0.0001
Lead*	-0.057	0.028	-2.060	0.045

* Statistically significant

Table 17. Associations between the BNBAS Abnormal Reflexes domain and second trimester average prenatal 3-phenoxybenzoic acid, dialkylphosphate metabolite, and heavy metal analyte levels

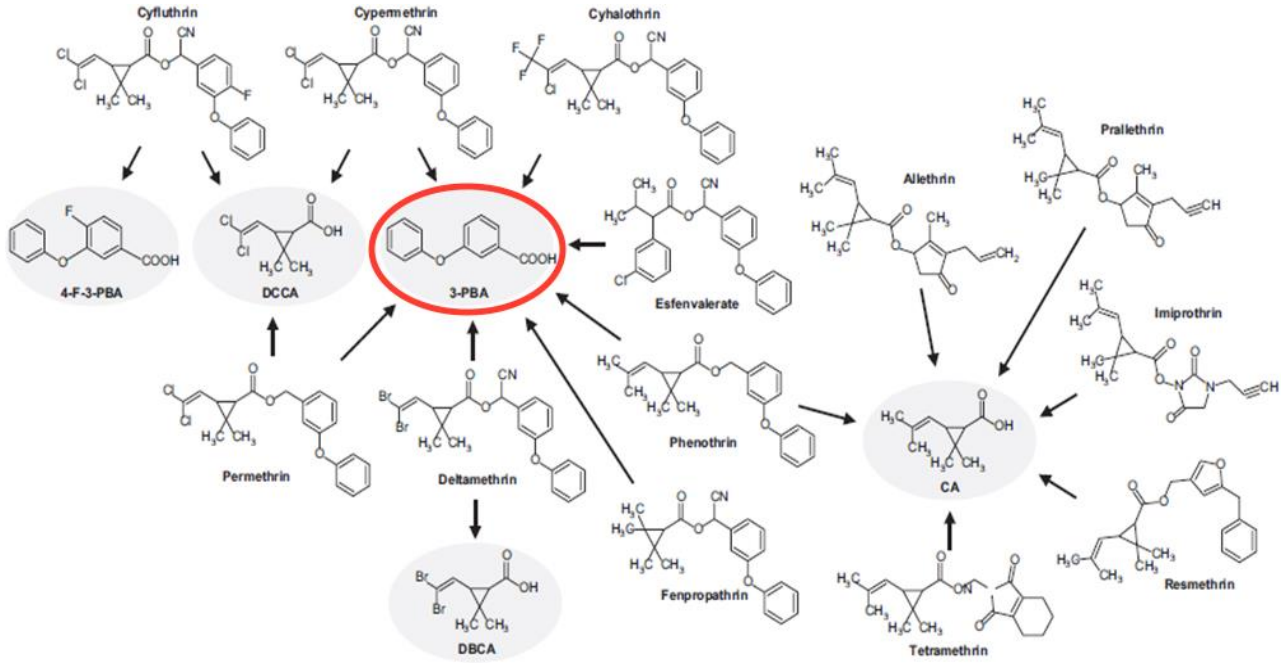
Coefficients	**Total Pregnancy[a] , N=54**		**First Trimester[a], N=17**	
	β	*p-value*	*β*	*p-value*
3-PBA	-0.30	0.41	-0.44	0.34
DAP	0.00	0.34	0.00	0.35
Arsenic	0.12	0.08	0.34	0.92
Mercury	-0.00	0.98	0.06	0.71
Lead	-0.01	0.34	-0.00	0.67
Cadmium	-0.02	0.97	-0.31	0.57

	Second Trimester[a] , N=30		**Third Trimester[a], N=53**	
	β	*p-value*	*β*	*p-value*
3-PBA	0.08	0.77	-0.08	0.77
DAP	0.00	0.43	-0.00	0.29
Arsenic	0.06	0.76	0.04	0.27
Mercury	0.00	0.96	0.04	0.73
Lead	-0.01	0.53	-0.00	0.76
Cadmium	-0.38	0.40	0.17	0.49



[a] Adjusted for urinary creatinine

Figure 2. Structures of some pyrethroid metabolites (Wu et al, 2011)



VIII. APPENDIX

1. Domains of Behavior Assessed by the Brazelton Neonatal Behavioral Assessment Scale

Domain	Description
Habituation	Ability to respond to and inhibit discrete stimuli while asleep
Orientation	Attention to visual and auditory stimuli and quality of overall alertness
Motor	Motor performance and quality of movement and tone
Range of State	A measure of infant arousal and state lability
Regulation of State	Ability to regulate state in the face of increasing levels of stimulation
Autonomic Stability	Signs of stress related to homeostatic adjustments of the central nervous system
Abnormal Reflexes	Number and type of abnormal primitive reflexes

2. Neonatal Behavioral Assessment Scale Items (Brazleton & Nugent 2011)

TABLE 1.1
Behavioral, supplementary and reflex items on the NBAS

Behavioral items	Supplementary items
Response Decrement to Light	Quality of Alertness
Response Decrement to Rattle	Cost of Attention
Response Decrement to Bell	Examiner Facilitation
Response Decrement to Tactile Stimulation of the Foot	General Irritability
Orientation Inanimate Visual	Robustness and Endurance
Orientation Inanimate Auditory	State Regulation
Orientation Inanimate Visual and Auditory	Examiner's Emotional Response
Orientation Animate Visual	
Orientation Animate Auditory	*Reflex items*
Orientation Animate Visual and Auditory	Plantar Grasp
Alertness	Babinski
General Tonus	Ankle Clonus
Motor Maturity	Rooting
Pull-to-Sit	Sucking
Defensive Movements	Glabella
Activity Level	Passive Movements – Arms
Peak of Excitement	Passive Movements – Legs
Rapidity of Build-up	Palmar Grasp
Irritability	Placing
Lability of States	Standing
Cuddliness	Walking
Consolability	Crawling
Self-Quieting	Incurvation (Gallant Response)
Hand-to-Mouth	Tonic Deviation of Head and Eyes
Tremulousness	Nystagmus
Startles	Tonic Neck Reflex
Lability of Skin Color	Moro
Smiles	

3. Neonatal Behavioral Assessment Scale Scoring Form (Brazleton & Nugent 2011)

APPENDIX

NBAS scoring form

Name _____ Sex _____ Date of birth _____

Gestational age ____ Weight ____ Height ____ Head circumference _____

Mode of delivery _____ Length of labor _____ Apgar scores _____

Parity _____ Type of feeding _____ Examiner _____

Date of examination _____

	Infant behavior									Comments
HABITUATION	9	8	7	6	5	4	3	2	1	
Response Dec.—Light	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____
Response Dec.—Rattle	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____
Response Dec.—Bell	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____
Response Dec.—Foot	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____
SOCIAL—INTERACTIVE	9	8	7	6	5	4	3	2	1	
Animate Visual	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____
Animate Vis. + Aud.	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____
Inanimate Visual	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____
Inanimate Vis. + Aud.	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____
Animate Auditory	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____
Inanimate Auditory	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____
Alertness	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____
MOTOR SYSTEM	9	8	7	6	5	4	3	2	1	
General Tone	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____
Motor Maturity	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____
Pull-to-Sit	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____
Defensive	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____
Activity Level	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____

STATE ORGANIZATION	9	8	7	6	5	4	3	2	1	
Peak of Excitement	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____
Rapidity of Build-up	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____
Irritability	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____
Lability of States	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____

STATE REGULATION	9	8	7	6	5	4	3	2	1	
Cuddliness	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____
Consolability	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____
Self-Quieting	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____
Hand-to-Mouth	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____

AUTONOMIC SYSTEM	9	8	7	6	5	4	3	2	1	
Tremulousness	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____
Startles	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____
Lability of Skin Color	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____
Smiles	❑									_____

SUPPLEMENTARY ITEMS	9	8	7	6	5	4	3	2	1	
Quality of Alertness	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____
Cost of Attention	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____
Examiner Facilitation	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____
General Irritability	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____
Robustness/Endurance	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____
State Regulation	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____
Examiner's Emot. Resp.	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____

REFLEXES	0	1	2	3	Asym	Comments
Plantar Grasp	❑	❑	❑	❑	❑	_____
Babinski	❑	❑	❑	❑	❑	_____
Ankle Clonus	❑	❑	❑	❑	❑	_____
Rooting	❑	❑	❑	❑	❑	_____
Sucking	❑	❑	❑	❑	❑	_____
Glabella	❑	❑	❑	❑	❑	_____
Passive Resist.—Legs	❑	❑	❑	❑	❑	_____
Passive Resist.—Arms	❑	❑	❑	❑	❑	_____
Palmar Grasp	❑	❑	❑	❑	❑	_____
Placing	❑	❑	❑	❑	❑	_____
Standing	❑	❑	❑	❑	❑	_____
Walking	❑	❑	❑	❑	❑	_____
Crawling	❑	❑	❑	❑	❑	_____
Incurvation	❑	❑	❑	❑	❑	_____
Tonic Dev. Head/Eyes	❑	❑	❑	❑	❑	_____

Nystagmus	❑ ❑ ❑ ❑ ❑	_____
Tonic Neck Reflex	❑ ❑ ❑ ❑ ❑	_____
Moro	❑ ❑ ❑ ❑ ❑	_____

SUMMARY: INFANT

Strengths Concerns

SUMMARY: PARENT(S)

Strengths Concerns

RECOMMENDATIONS FOR CAREGIVING:

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4. Neonatal Behavioral Assessment Scale Seven Cluster Scoring Method (Brazleton & Nugent 2011)

TABLE 4.1
NBAS seven-cluster scoring criteria

Cluster item	Scoring
Habituation	
Light	Raw score
Rattle	Raw score
Bell	Raw score
Pin-prick	Raw score
Orientation	
Inanimate Visual	Raw score
Inanimate Auditory	Raw score
Inanimate Visual-Auditory	Raw score
Animate Visual	Raw score
Animate Auditory	Raw score
Animate Visual-Auditory	Raw score
Alertness	Raw score
Motor	
Tonus	Recode: 9/1=1; 8/2=2; 7/3=3; 4=4; 6=5; 5=6
Maturity	Raw score
Pull-to-Sit	Raw score
Defense	Raw score
Activity	Recode: 9/1=1; 8/2=2; 7/3=3; 4/6=4; 5=5
Range of State	
Peak of Excitement	Recode: 9/1=1; 8/2=2; 3=3; 7/3=4; 6/4=5
Rapidity of Build-up	Raw score
Irritability	Recode: 9/1=1; 8=2; 7=3; 6=4; 5=5; 2/3/4=6
Lability of State	Recode: 9=1; 7/8=2; 5/6=3; 3/4=4; 1/2=5
Regulation of State	
Cuddliness	Raw score
Consolability	Raw score
Self-Quieting	Raw score
Hand-to-Mouth	Raw score
Autonomic Stability	
Tremors	Recode: Invert: 9=1 (1=9); 8=2 (2=8); etc.
Startles	Recode: If 1, drop; otherwise invert 2–9 on 8-point scale
Skin Color	Recode: 1/9=1; 2/8=2; 3/7=3; 4/6=4; 5=5
Reflexes	An abnormal score is defined as 0, 1 or 3 for all reflexes except clonus, nystagmus and TNR where 0, 1 and 2 are normal and 3 is abnormal. Reflex score = total number of abnormal reflex scores