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Prenatal Organophosphate Pesticide Exposure and Neurodevelopmental Outcomes in a Thai Agricultural Birth Cohort

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By

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B.S.
Northeastern University
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Abstract

Prenatal Organophosphate Pesticide Exposure and Neurodevelopmental Outcomes in a Thai Agricultural Birth Cohort

By Emilia Matthews

Recent research indicates low-level exposure to organophosphate (OP) pesticides during critical periods of development, particularly in utero, can have lasting neurotoxic effects. This study aimed to assess the relation between in utero OP pesticide exposure and neurologic integrity at birth, as measured by seven clusters on the Brazelton Neonatal Behavioral Assessment Scale (BNBAS). Trimester-resolved concentrations of urinary dialkylphosphate (DAP) metabolites (including diethylphosphates [DEPs] and dimethyl phosphates [DMPs]) of OP pesticides were measured to assess exposure to fetuses of tangerine farmworkers in Northern Thailand participating in a 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). Results from the SAWASDEE cohort indicate these infants are more highly exposed in utero to OP pesticides and perform less optimally on the BNBAS than two comparable U.S. birth cohorts, the **C**enter for the **H**ealth **A**ssessment of **M**others **A**nd **C**hildren of **S**alinas (CHAMACOS) cohort of California and the Mount Sinai Children's Environmental Health Cohort of New York. We observed inverse associations between total pregnancy ΣDAP and ΣDEP with Orientation (β = -5.10, 95% CI: -9.53, -0.68; β=-3.93, 95%CI: -7.86, 0.01, respectively) and Motor clusters (β = -2.92, 95% CI: -5.65, -0.19; β=-2.46, 95%CI: -4.87, -0.047, respectively), indicating poorer performance with increasing DAP metabolite concentrations. Second trimester metabolite concentrations showed stronger associations than total pregnancy metabolite concentrations (Orientation: ΣDAP β = -5.69, 95% CI: -9.69, -1.69; ΣDEP β = -4.66, 95% CI: -8.31, -1.01; Motor: ΣDAP β = -3.49, 95% CI: -5.78, -1.20; ΣDEP β = -3.06, 95% CI: -5.13, -0.99). A positive association between first trimester ΣDAP and ΣDMP with the Abnormal Reflex cluster was also observed (β = 0.91, 95% CI: 0.29, 1.61; β=0.99, 95%CI: 0.26, 1.71, respectively) indicating poorer performance with increasing DAP metabolite concentrations. These results are suggestive of a detrimental association between prenatal OP pesticide exposure and neurobehavioral functioning at birth, particularly on measures of attention, motor function, and abnormal reflexes. This study is the first to examine the impact of trimester-specific exposure to OP pesticides on neurodevelopment at birth using several measures of an exposure biomarker in a highly exposed agricultural population.

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I. BACKGROUND & SIGNIFICANCE

Organophosphate Pesticides

Organophosphate (OP) pesticides are used abundantly worldwide despite the fact that they are known to have adverse effects on human health. In Thailand, pesticide use has increased four-fold in the past decade and OP pesticides are now the most abundantly used class of pesticides for agricultural purposes (Panuwet et al., 2012). Approximately 40% of Thai women are employed in agriculture, and many continue to work during pregnancy (Kongtip et al., 2013; The World Bank, 2013). Recently, animal and human studies have raised considerable concern regarding the potential for prenatal exposure to OP pesticides to impact fetal development, and specifically neurodevelopment (Aldridge, Meyer, Seidler, & Slotkin, 2005; Munoz-Quezada et al., 2013).

The neurotoxic properties of OP pesticides at high doses are well known. Like nerve agents, OP pesticides act by inhibiting the enzyme acetylcholinesterase from breaking down the neurotransmitter acetylcholine, resulting in disruption of the nervous system. Recent research has shown that adverse effects can occur at levels lower than those required to cause acute toxicity by mechanisms other than cholinesterase inhibition (Rauh et al., 2006; Rauh et al., 2012). This means that more subtle effects resulting from lower-level exposures may go undetected, and current risk assessments based on cholinesterase inhibition as the indicator of toxicity may be inadequate for protecting children's neurodevelopment (Rauh et al., 2006; Rauh et al., 2012). Even low-level exposure to OP pesticides during critical periods of development,

such as gestation and early childhood, can have lasting non-acute neurotoxic effects (Aldridge et al., 2005; Rauh et al., 2012).

Prenatal Exposure

Prenatal exposure is particularly concerning given the inherent vulnerability of the fetus to exposures that occur during critical windows of development. Additional susceptibility results from the fact that OP pesticides can cross the placenta and enter the fetal bloodstream (Landrigan PJ, 1999). Because metabolic pathways are immature during gestation, the fetus is less able to detoxify harmful chemicals, including OP pesticides, leading to increased vulnerability (Landrigan PJ, 1999). Some researchers also suspect that the half-life of OP pesticides in the fetus may be longer than in adults as a result of reduced clearance mechanisms (Whyatt et al., 2004). Any effects on development resulting from exposure to toxicants during this time period can be long lasting and irreversible (Barone, Das, Lassiter, & White, 2000; Landrigan PJ, 1999).

Neurodevelopmental Effects

A recent systematic review of studies examining the association between OP pesticide exposure and neurodevelopment in children reported that 26 out of the 27 studies reviewed provided evidence that exposure to OP pesticides, especially prenatally, is a risk factor for poor neurodevelopment (Munoz-Quezada et al., 2013). Evidence of a positive dose-response relationship, indicating increased adverse neurodevelopmental effects with higher levels of OP exposure, was found in 11 out of 12 studies evaluating dose-response (Munoz-Quezada et

al., 2013). Four birth cohort studies conducted in the United States have evaluated the effects of prenatal pesticide exposure on fetal development and have found OP pesticide exposure to be associated with decreased birth weight and length, shortened gestation, and decreased head circumference (Berkowitz et al., 2004; Engel et al., 2007; Rauch et al., 2012; Whyatt et al., 2004; Young et al., 2005). In addition, studies from three of these cohorts have found prenatal and to a lesser extent early childhood OP pesticide exposure to be associated with adverse neurodevelopmental outcomes extending into the early school years, including: decreased IQ, delays in psychomotor and mental development, increased symptoms of pervasive developmental disorders, and increased attention problems including ADHD (Bouchard et al., 2011; Engel et al., 2011; Eskenazi et al., 2007; Marks et al., 2010; Rauh et al., 2006; Rauh et al., 2012). The weight of evidence is now strong, indicating that prenatal exposure to OP pesticides can have adverse developmental consequences for children. However, gaps in our knowledge exist regarding the timing of exposure and when exactly it is most harmful; these knowledge gaps are hampering the ability for these new and compelling data to be incorporated into the risk assessment process.

Dialkyl phosphate Metabolites

Until recently, most studies on this topic have lacked the temporally-resolved prenatal exposure data needed in order to better understand whether OP pesticide exposure affects neurodevelopment through repeated exposures over time or short-term exposures during specific critical windows of development (Munoz-Quezada et al., 2013). Measurement of urinary dialkyl

phosphate (DAP) metabolites is one of the most widely used, biologically-based exposure assessment techniques for assessing exposure to OP pesticides (Munoz-Quezada et al., 2013). Six DAP metabolites are commonly quantified to assess OP pesticide dose, including three dimethyl phosphates [dimethylphosphate (DMP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP)] and three diethyl phosphates [diethylphosphate (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP)], as shown in ***Figure 1*** (Barr et al., 2004; Munoz-Quezada et al., 2013).

Since each of these metabolites may correspond to one or more OP pesticides, the measurement of DAP metabolites is used to provide information on cumulative exposure to OPs as a class of pesticides (***Figure 2***) (Barr et al., 2004). The measurement of urinary DAP metabolites represents exposure to parent pesticide compounds as well as preformed metabolites in the environment and may therefore overestimate exposure (Munoz-Quezada et al., 2013). However, these biomarker measurements are often the preferred method used for OP pesticide exposure assessment, and they are certainly an improvement over previous methods based on self-reported or ecologic measures of exposure.

While DAP metabolite concentrations are assessed in the U.S. general population by NHANES to establish reference values for comparison, biomonitoring of exposure is just beginning to be conducted in Thailand in a few small pilot studies (Hanchenlaksh, Povey, O'Brien, & de Vocht, 2011; Kongtip et al., 2013; Panuwet et al., 2012). Currently no nationally representative reference value for exposure to OP pesticides exists there. 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 (Hanchenlaksh et al., 2011; Kongtip et al., 2013; Panuwet et al., 2012).

SAWASDEE Birth Cohort

While a limited number of studies have been conducted in Thailand to assess pesticide exposure, only two pilot birth cohort studies have been conducted in the region: our **S**tudy of **A**sian **W**omen **a**nd their off**S**pring's **D**evelopment and **E**nvironmental **E**xposures (SAWASDEE[1]) conducted in a densely agricultural region of northern Thailand, and the Mahidol Study conducted in northeastern, lower north and western Thailand (Kongtip et al., 2014). Our SAWASDEE study, however, is the first birth cohort study in Thailand or elsewhere to have temporally-resolved exposure data, thus capturing monthly and trimester-specific exposures.

All of the women enrolled in the SAWASDEE pilot birth cohort work in agriculture as tangerine farmworkers in Fang District, Chiang Mai Province of Northern Thailand and are occupationally exposed to OP pesticides. Some exposure may also occur through residential use of OP pesticides. 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.

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

Brazelton Neonatal Behavioral Assessment Scale

The present study will assess the effect of prenatal OP pesticide exposure, as measured by maternal urinary DAP metabolites, on neurodevelopment at birth as measured by the Brazelton Neonatal Behavioral Assessment Scale (BNBAS). The BNBAS is a standardized, well-validated test for assessing neurodevelopment in infants from birth to two months of age, without the need for any culturally-specific modifications or validation (Brazelton & Nugent, 2011). The BNBAS evaluates a set of 18 reflexes and 28 behavioral items in infants to assess seven domains of behavior, described in ***Table 1***, including: Habituation, Orientation, Motor, Range of State, Regulation of State, Autonomic Stability, and Abnormal Reflexes (Engel et al., 2007; Young et al., 2005).

To date, only two U.S. studies have assessed the effect of prenatal OP exposure on neurodevelopment at birth (Engel et al., 2007; Young et al., 2005). Both studies used the BNBAS, which was administered shortly after birth. Higher scores on BNBAS domains are associated with more optimal functioning, except for the domain of abnormal reflexes. A higher score for the reflex domain indicates less optimal functioning, with two or greater observed abnormal reflexes warranting further clinical evaluation and the need for possible intervention (Engel et al., 2007; Young et al., 2005). Young et al. (2005) measured DAP metabolites in two maternal urine samples collected during pregnancy from Latina women participating in the **C**enter for **H**ealth **A**ssessment of **M**others **a**nd **C**hildren of **S**alinas (CHAMACOS) longitudinal birth cohort study in California. Young et al. (2005) reported a significant positive association

between abnormal reflexes and total DAP metabolites (β=0.23, 95% CI 0.05, 0.41), dimethyl phosphate metabolites (β=0.18, 95% CI 0.02, 0.34), and diethyl phosphate metabolites (β=0.22, 95% CI 0.04, 0.40). Inverse associations were reported for Orientation, Motor, Regulation of State, and Autonomic Stability clusters with DAP metabolites, particularly DEP metabolites, but the results were not statistically significant.

Similarly, Engel et al. (2007) measured DAP metabolites in maternal urine during the third trimester of pregnancy in women participating in a multiethnic pregnancy cohort in New York City, the Mount Sinai Children's Environmental Health Cohort, and found a significant positive association between summed diethyl phosphate (ΣDEP) metabolites and increased abnormal primitive reflexes in the infants at birth (RR = 1.49, 95% CI 1.12, 1.98). Positive associations were also reported for summed dimethyl phosphate (ΣDMP) metabolites and total DAP metabolites, but the associations were not significant. Inverse associations were reported for DEP and total DAP metabolites with the domains of Orientation, Regulation of State, and Autonomic Stability, indicating less optimal functioning with increasing exposure to OP pesticides, but these results were not statistically significant (Engel et al., 2007).

Based on these two studies, it appears that increasing total DAP, ΣDMP, and ΣDEP during pregnancy are associated with increased abnormal reflexes but not other measures of behavioral performance at birth as measured by the BNBAS. However, both studies were hampered by infrequent and inconsistent measurement of exposure biomarkers during pregnancy. Engel et al. (2007) relied on a single urine sample collected during the third trimester, while Young

et al. (2005) relied on two samples, collected at approximately 14 and 26 weeks during pregnancy, that were poorly (and not significantly) correlated with one another (Engel et al., 2007; Young et al., 2005). The present study will investigate the relation of prenatal exposure to OP pesticides, measured multiple times during each trimester of pregnancy, on infants' neurodevelopment assessed at birth by the BNBAS in the SAWASDEE pilot birth cohort.

II. METHODS

Participants and Recruitment

All study protocols were reviewed and approved by the Institutional Review Board of Emory University and the Ethic Boards of Chiang Mai University and the Thai Ministry of Health. The SAWASDEE study is a longitudinal pilot birth cohort of farmworker women and neonates residing in the Chiang Mai Province of northern Thailand. Between March 2011 and February 2012, 59 pregnant women were recruited into the cohort during their first prenatal visit to the antenatal clinic at Fang Hospital located in northern Thailand. Inclusion criteria were: 1) aged 18-40 years; 2) Thai identification card permitting hospital and antenatal clinic access; 3) Thai as primary language at home; 4) residence in their regional district for ≥ 6 months and planned residence at least 1 month after delivery; 5) good general health (i.e. no major medical conditions such as hypertension, diabetes, thyroid disease, HIV); 6) consumption of fewer than two alcoholic beverages per day and no use of illegal drugs. The Thai identification card allowed each pregnant woman a minimum of

one monthly prenatal visit to an OB/GYN. These women were followed longitudinally at each prenatal and postnatal visit until about three days after delivery. The participation rate was high (59/59 or 100%). Three participants were lost to follow up or were excluded due to spontaneous abortion, resulting in an overall retention rate of 95%.

Participants were administered a comprehensive questionnaire at the time of enrollment, at 28 weeks and 36 weeks gestation which included demographic data such as maternal age, maternal education, household income, and maternal occupation. Questionnaire data also included information on pesticide-related activities, knowledge of pesticide hazards and safe-use practices, and maternal health and lifestyle factors, including smoking and alcohol consumption during pregnancy. Additional information was abstracted from medical records, including infant sex, birth weight, birth length, gestational age, head circumference, APGAR scores and pregnancy or delivery complications.

Exposure Assessment

Spot urine samples were collected at each prenatal visit at the antenatal clinic, using 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. Dialkyl phosphate (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 elsewhere (Prapamontol et al., 2013). This method showed a relative recovery range of 94.4 - 119% and relative

standard deviations (RSD) of less than 20%. The limits of detection were reported from 0.1 ng/mL urine to 2.5 ng/mL urine for all six common DAP metabolites (Prapamontol et al., 2013).

Maternal urine samples were collected at multiple timepoints throughout pregnancy, with an average of 8 samples for each woman, as well as once postnatally. Given the short half-life of OPs, metabolite levels measured in postnatal urine samples reflect postpartum rather than in utero exposure. Therefore, postnatal samples were not included in the analyses.

Diethyl and dimethyl phosphate metabolites were converted to their molar equivalents by dividing by their respective molecular weights and then were summed on a molar basis (nanomoles/liter, nmol/L, or nM) using ***Equation 1***.

Equation 1.

$$\boldsymbol{\Sigma DEP} = \frac{[DEP]}{149\frac{ng}{nmol}} + \frac{[DETP]}{165\frac{ng}{nmol}} + \frac{[DEDTP]}{181\frac{ng}{nmol}} = \frac{nmol}{mL} x \frac{1000mL}{1L} = nM$$

$$\boldsymbol{\Sigma DMP} = \frac{[DMP]}{125\ ng/nmol} + \frac{[DMTP]}{141\ ng/nmol} + \frac{[DMDTP]}{157\ ng/nmol} = \frac{nmol}{mL} x \frac{1000mL}{1L} = nM$$

$$\boldsymbol{\Sigma DAP} = \boldsymbol{\Sigma DEP} + \boldsymbol{\Sigma DMP}$$

This produced summary measures for total diethyl phosphates (ΣDEP) and total dimethyl phosphates (ΣDMP), respectively, and together to obtain total dialkyl phosphates (ΣDAP) yielding three summary measures for each urine sample, as shown in ***Figure 3***. Each participant's samples were averaged across

trimesters to create exposure measures for each trimester of pregnancy as well as an overall pregnancy average for each of the three summary measures.

Outcome Assessment

In this cohort, the BNBAS was administered by a test-certified nurse once to each infant within three days of birth following the BNBAS protocol. The BNBAS was administered to 55 infants in total, however some infants were missing scores for certain domains. Typically, the BNBAS is scored using the Lester et al. (1982) seven-cluster scoring method, which divides the 28 behavioral items and 18 reflexes into seven domains (or clusters): habituation, orientation, motor performance, range of state, regulation of state, autonomic stability, and abnormal reflexes (Brazelton & Nugent, 2011). Each of the 28 behavioral items is scored on a nine-point scale, with three to six items making up each of the six domains. The seventh domain is comprised of the 18 reflex items, which are each scored on a four-point scale indicating the degree of abnormality.

Cluster scores for the six domains concerning the 28 behavioral items are calculated by recoding the original BNBAS items when necessary so that higher scores represent more optimal functioning. The recoded individual items within each domain are averaged to obtain an average score for each domain, or summed to obtain a summary score for each domain. For the reflex domain, a score of two is considered normal while a score of 0, 1 or 3 is considered abnormal for 15 out of the 18 reflexes. For the remaining three reflexes, 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,

with a higher count indicating less optimal functioning. Additional details on the scoring method are included in ***Appendix 1.***

Aims and Hypotheses

AIM 1: To describe prenatal exposure to OP pesticides in the SAWASDEE cohort.

- **Hypothesis:** Maternal DAP concentrations will vary over the course of pregnancy, indicating the necessity of multiple measures of exposure.

AIM 2: To assess the effect of prenatal exposure to organophosphate pesticides on neurologic integrity at birth as measured using the BNBAS.

- **Hypothesis 1a:** Maternal DAP metabolites will be inversely associated with attention parameters as measured by the BNBAS.
- **Hypothesis 1b:** Maternal DAP metabolites will be positively associated with abnormal reflexes as measured by the BNBAS.

Data Analysis

Data were analyzed using SAS software, version 9.3 (SAS Institute, Inc., Cary, North Carolina). Univariate analyses were conducted to describe all exposure and outcome measures, and to assess skewness in these variables. Correlation measures were used when appropriate to assess bivariate relationships between the exposure variables and the outcome measures, as well as with potential confounders.

To assess the relation between DAP metabolite levels and neonatal performance on the BNBAS, separate logistic regression models were fit for each

of the seven domains. For each of the six behavioral domains, the averaged scores were dichotomized at the median. For the reflex domain, the count of abnormal reflexes was dichotomized at greater than or equal to 2, since observing 2 or more abnormal reflexes is considered clinically significant and often signifies a need for further neurologic examination (Engel et al., 2007). Because of skewness in the distributions of the DAP metabolite concentrations, all analyses were performed on $\log_{10}$ transformed concentrations. Total dialkylphosphate metabolites (ΣDAP), total diethylphosphate metabolites (ΣDEP), and total dimethylphosphate metabolites (ΣDMP) were assessed as independent predictors, with first, second, or third trimester specific averages or total pregnancy averages included independently in the models.

In addition, separate linear regression models were fit for six of the seven domains, excluding abnormal reflexes. For each of the six behavioral domains, the summary scores were treated as continuous variables. As with the logistic models, ΣDAP, ΣDEP, and ΣDMP were assessed as $\log_{10}$ transformed predictors, with first, second, or third trimester specific averages or total pregnancy averages included independently in the models. Poisson regression models were fit for abnormal reflexes because the data were derived from counts rather than scores using the same exposure variables (ΣDAP, ΣDEP, ΣDMP) as predictors.

Covariates considered as potential confounders based on previous literature included maternal age, maternal education, household income, and maternal pre-pregnancy BMI. 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 β coefficient for the exposure predictors when comparing crude estimates to adjusted estimates. Because gestational age at delivery and birth weight are potential causal intermediates in the relationship between OP exposure and neurodevelopmental outcomes, they were not evaluated for confounding. However, two sensitivity analyses were conducted excluding either preterm or low birth weight infants.

III. RESULTS

Demographic Data

Table 2 presents demographic characteristics of the SAWASDEE pilot birth cohort. Participants in this study were predominantly young women born in Burma (64.3%) and were of Thai Yai ethnicity (60.7%), with low educational attainment and low household income. The majority were unmarried but living as such (91.1%). Most women were enrolled during the second trimester of pregnancy, with an average gestational age at enrollment of 15 weeks, while only 13 (23.2%) women were enrolled during the first trimester. The majority of women were enrolled during the rainy season from May to October (60.7%). Maternal pre-pregnancy body mass index (BMI) was normal for the majority of participants (80.0%). Most women in the SAWASDEE cohort delivered term

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

infants (85.7%) of normal birth weight (87.5%). The SAWASDEE cohort included eight preterm (<37 weeks gestation) infants and eight low birth weight (<2500 g) infants, four of which were both preterm and low birth weight.

Exposure Distribution

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, with a range of 5 to 13. Median metabolite levels for the average of all pregnancy measurements of ΣDAP, ΣDEP and ΣDMP in maternal urine were 187.8, 152.9 and 24.6 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, as shown in ***Figure 4***. First trimester urinary metabolite levels showed the most variation compared to second and third trimester levels. ΣDEP were detected at much higher concentrations and showed more variation compared to ΣDMP, as indicated by a wider range and higher standard deviation, although this is most likely an artifactual result of the lower frequency of detection of several of the DMP metabolites.

Outcome Distribution

Table 4 presents sample mean and median values, standard deviations, and ranges for the summed measures of each of the seven cluster scores, which were used in the linear regression models, as well as the same descriptive data for the average summary measures of each of the seven clusters, which were used in

the logistic regression models. ***Table 5*** also presents the highest possible score for each summed measure on six of the seven clusters, excluding abnormal reflexes, since each cluster incorporated a different number of items each scored on a 9-point scale. The Orientation cluster exhibited the most variation, with the widest range and highest standard deviation, but also the second highest mean and median when taking into account the larger number of items used to obtain this score, indicating better performance overall on this cluster. Out of all six behavioral clusters, excluding the abnormal reflex cluster, infants performed most optimally on the Habituation cluster, as indicated by its higher mean and median when taking into account the number of items used to obtain this score. The Range of State cluster exhibited the least variation, but also the lowest overall mean and median indicating poorer performance on this cluster overall. In terms of the average summary measures for the six clusters, the medians for the Motor, Range of State, and Regulation of State clusters are less than or equal to 5, which is the point at which scores become more or less optimal on the 9-point scale. For these six clusters, the average scores were dichotomized at the medians for the logistic regression analyses. For the Abnormal Reflexes cluster, most infants exhibited one abnormal reflex on average, with a range of 0 to 6, with more reflexes indicating less optimal performance. This cluster was dichotomized at greater than or equal to 2 for the logistic regression models, since this is the point at which further evaluation may be indicated (Brazelton & Nugent, 2011).

Logistic Regression Analysis

Tables 1-4 (included in ***Appendix B***) present adjusted odds ratios and 95% confidence intervals for each of the seven BNBAS cluster scores, regressed separately on total pregnancy and trimester specific averages of ΣDMP, ΣDEP, and ΣDMP measured during pregnancy. A significant association was found between increasing second trimester ΣDAP and odds of a less-optimal performance on the Orientation cluster of the BNBAS, with an odds ratio of 3.718 (95% CI: 1.073, 12.879). Second trimester ΣDEP levels largely drove this association, as indicated by an odds ratio of 3.095 (95% CI: 1.002, 9.557). No other significant associations between urinary metabolite levels and BNBAS clusters were observed.

Linear Regression Analysis

Tables 6 - 9 present results of the linear regression models, with adjusted regression coefficients and 95% confidence intervals for six of the BNBAS clusters, excluding abnormal reflexes, with total pregnancy, first trimester, second trimester, and third trimester exposures respectively. A significant association between increased total pregnancy ΣDAP and Orientation was observed (R^2=0.09, p=0.025) indicating less optimal performance on the Orientation cluster with increasing total pregnancy ΣDAP metabolite levels (***Table 6)***. Again this association was driven largely by ΣDEP rather than ΣDMP metabolite levels, indicated by a nearly significant association observed with ΣDEP (R^2=0.07, p=0.05) and a null association with ΣDMP (R^2=0.13, p=0.15). In addition, significant associations were observed between increased second

trimester ΣDAP and Orientation (R^2=0.14, p=0.006), and between increased second trimester ΣDEP and Orientation (R^2=0.12, p=0.004), again indicating poorer performance on the Orientation cluster with increasing ΣDAP and ΣDEP in the second trimester (***Table 8)***. Associations between the Orientation cluster score and metabolite levels were stronger for second trimester exposure measures compared to total pregnancy measures. The associations between total pregnancy ΣDAP and ΣDEP with the Motor cluster were also statistically significant, (R^2=0.09, p=0.036 and R^2=0.08, p=0.046, respectively), indicating poorer performance on the Motor cluster with increasing total pregnancy ΣDAP and ΣDEP (***Table 6***). Associations between second trimester ΣDAP and ΣDEP and the Motor cluster were also significant, (R^2=0.16, p=0.004 and R^2=0.15, p=0.005, respectively) and slightly stronger than the associations with total pregnancy metabolite levels, showing a similar pattern to the association with the Orientation cluster (***Table 8***). No other associations between urinary metabolite levels and BNBAS clusters were statistically significant.

Poisson Regression Analysis

Table 10 presents results of the Poisson regression models, with adjusted regression coefficients and 95% confidence intervals for abnormal reflexes. Associations between first trimester ΣDAP and ΣDMP and the Abnormal Reflex cluster were significant, (β=0.91, 95% CI: 0.20, 1.61 and β=0.99, 95% CI: 0.26, 1.71, respectively) indicating an increase in abnormal reflexes with increasing ΣDAP and ΣDMP metabolite levels. No other associations between urinary metabolite levels and the Abnormal Reflex cluster were statistically significant.

Sensitivity Analysis

Two sensitivity analyses were conducted, one excluding eight low birth weight infants and one excluding eight preterm infants. Both analyses resulted in an attenuation of the associations observed between total pregnancy ΣDAP or ΣDEP with NBAS sum scores for the orientation and motor clusters. Observed associations between second trimester ΣDAP or ΣDEP with these clusters was also attenuated. Likewise, Poisson regression analyses assessing the association between first trimester ΣDAP and ΣDMP were attenuated when low birth weight or preterm infants were excluded. After excluding low birth weight or preterm infants, these previously significant results were no longer statistically significant.

Two-sample t-tests revealed significant differences between low birth weight and normal birth weight infants in the mean sum scores for orientation and motor clusters as well as mean count of abnormal reflexes, but not for total pregnancy ΣDAP metabolites. Statistically significant differences in the means were also observed between preterm versus term infants for these three clusters.

IV. DISCUSSION

Interpretation of Results

Only two studies to date have investigated the effect of prenatal exposure to OP pesticides on neurologic integrity at birth as measured by the BNBAS (Engel et al., 2007; Young et al., 2005). Both studies relied on only one or two maternal urine samples to characterize infants' in utero exposure for the duration of pregnancy, and were therefore unable to assess the effect of trimester specific

exposure. Exposure assessment based on one or two biological sample is less representative of long-term exposure than serial measurements (Bouchard et al., 2011). The present study collected an average of 8 maternal urine samples, with as many as 13 samples collected from some participants. As a result, we were able to characterize infants' exposure to OP pesticides over the course of pregnancy more accurately than previous studies, and we were able to independently assess the effect of trimester specific exposure on each of the BNBAS outcomes. Exposure distributions of DAP metabolites in the present study are much higher than those reported by Young et al. (2005) for the CHAMACOS cohort and Engel et al. (2007) for the Mount Sinai Children's Environmental Health Cohort, as shown in ***Figure 5***.

Compared to the CHAMACOS cohort, infants in the SAWASDEE cohort performed less optimally on several clusters, including Orientation, Motor, Range of State, Regulation of State, and Autonomic Stability, as indicated by lower average cluster scores, shown in ***Figure 6***. The SAWASDEE cohort performed equally well on the Habituation cluster, and had a lower mean for the Abnormal Reflexes cluster.

Results of analyses based on this pilot birth cohort of 56 neonates born to Thai agricultural workers are suggestive of adverse neurodevelopmental effects of in utero OP pesticide exposure as measured by DAP metabolites, particularly in regards to attention, motor performance and primitive reflexes. We found an association between second trimester ΣDAP metabolite levels and increased odds of sub-optimal performance on the Orientation cluster of the BNBAS. This association was driven largely by DEP metabolites. This association was also

observed using linear regression, indicating less optimal performance on the Orientation cluster with increasing total pregnancy ΣDAP metabolite levels, as well as second trimester ΣDAP metabolite levels. DEP metabolite levels drive these associations as well.

We also observed associations between total pregnancy ΣDAP and ΣDEP as well as second trimester ΣDAP and ΣDEP with the Motor cluster, indicating poorer performance on this cluster with increasing DAP metabolite levels. The pattern of associations observed with both the Orientation cluster and the Motor appear similar, with DEP metabolites driving the associations, and second trimester metabolite levels showing slightly stronger associations than total pregnancy metabolite levels.

Lastly, we observed associations between first trimester ΣDAP and ΣDMP metabolite levels with the Abnormal Reflexes cluster, indicating an increase in the number of observed abnormal reflexes with increasing metabolite levels.

We used summed BNBAS scores instead of average BNBAS scores in our linear regression analyses, therefore we are unable to directly compare our results with those reported by Engel et al. (2007) and Young et al. (2005). However, our results show a similar pattern for the Orientation and Motor clusters. Young et al. (2005) and Engel et al. (2007) both reported inverse associations between average BNBAS cluster scores for Orientation and Motor with increased total DAPs and DEPs, based on measures at 14 and 26 weeks, or during the third trimester, respectively. Both Young et al. (2005) and Engel et al. (2007) found significant positive associations between a count of abnormal reflexes and total DAPs, DEPs, and DMPs. In the SAWASDEE cohort, we

observed significant positive associations for first trimester DAPs and DMPs only, which is slightly surprising because of the small number of women who provided first trimester samples (N=13). The association between abnormal reflexes and DAP metabolite levels did not reach significance for total pregnancy measures, nor for second or third trimester measures.

While the associations observed in two previous U.S. birth cohorts were non-significant, the patterns indicating adverse associations between specific BNBAS outcomes and prenatal OP exposure are the same as what we observed in the SAWASDEE Thai birth cohort. While the pattern we observed for these outcomes is in agreement with our *a priori* hypothesis of a detrimental effect of OP exposure on neurobehavioral functioning at birth, it is possible that these associations arose as a result of multiple testing.

To further support our findings, however, is the biological plausibility of effects during specific trimesters. Neuronal pathways that control orientation and refined motor skills are mostly developed during the second trimester. Perturbations or insults during the formation of these pathways can adversely affect their functioning after birth. Similarly, 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, again consistent with our findings (Rice & Barone, 2000).

Limitations

Organophosphate pesticides have short half-lives, and our results indicate that metabolite levels vary considerably throughout pregnancy. This indicates

that exposure to OP pesticides may not be fully captured by our current exposure assessment, particularly during specific critical windows of brain development, despite our attempt to observe this variation by measuring maternal metabolite levels at multiple time points throughout pregnancy. Only thirteen women provided urine samples during first trimester of pregnancy, which limited our ability to accurately investigate associations between BNBAS outcomes and first trimester OP exposure due to this small sample size, as indicated by wide confidence intervals. While exposure misclassification may have occurred as a result of variation in exposure measures and a lack of first trimester samples for many women, it is likely to be non-differential with respect to the outcome, and therefore would result in bias toward the null. Exposure data were very skewed due to a wide variation in exposure between mothers in our cohort, such that all exposure measures were $\log_{10}$-transformed. Even after correcting for skewness, DMP measures remained skewed, likely due to a high number of samples with non-detectable levels of DMP.

In addition, the measurement of urinary DAP metabolites represents exposure to parent pesticide compounds as well as preformed metabolites in the environment and may therefore overestimate exposure. Lastly, this method of exposure assessment relies on measuring maternal metabolite levels as a proxy for exposure to the fetus in utero. Even so, these biomarker measurements are currently the preferred method used for OP pesticide exposure assessment.

The ability of the BNBAS administered at birth to predict later neurological development is largely unknown, particularly when only a single assessment occurs. Therefore, it is possible that outcome misclassification may

have occurred due to the reliance on a single outcome assessment since the NBAS was administered only once to each infant shortly after birth. However, any outcome misclassification that occurred is likely to be non-differential with respect to exposure, and therefore it is expected that this would result in bias toward the null.

In this study sample, the BNBAS average clusters scores for three specific clusters are concerning, as indicated by median cluster scores less than 5 on a 9-point scale, which signifies less optimal neurobehavioral functioning. In the SAWASDEE cohort, the medians for the Motor, Range of State, and Regulation of State clusters were 5.0, 4.0 and 4.0 respectively. In logistic regression analyses, the median cluster scores were used as the point at which scores were dichotomized, dividing the scores into two categories of less optimal functioning and more optimal functioning. The median was chosen as the cut-point instead of a score of 5.0 because not enough infants scored a 5.0 or above on some clusters. As a result, some misclassification of the outcome may have occurred since some infants with less optimal scores were thereby categorized as having a more optimal score. However, this would likely result in bias toward the null and an attenuation of the effect. Linear regression may be a preferred method of analysis over logistic regression, as indicated by narrower confidence intervals, particularly for first trimester analyses, and better fit observed for the linear models. However, it is reassuring that results from the two analyses were in agreement.

While some important confounders were considered in the present analysis, including maternal education, household income, maternal pre-

pregnancy BMI and maternal age, there are some important confounders that we were unable to assess. Most importantly, we were unable to assess parental IQ and exposure to other known neurotoxicants, such as lead, tobacco smoke, alcohol, and polychlorinated biphyenyls (PCBs). In addition, our population was largely homogenous on measures of maternal education and household income, which reduces the potential for uncontrolled confounding of these covariates, but as a result our control of these confounders may not be adequate and some residual confounding may be present. In addition, when low birth weight or preterm infants were excluded, our results were attenuated.

Despite these limitations, this study is the first to examine the impact of trimester specific exposure to organophosphate pesticides on neurodevelopment at birth using several measures of an exposure biomarker in a highly exposed agricultural population. Further investigation is needed to confirm these results.

V. CONCLUSION

Summary

Results from the SAWASDEE pilot birth cohort indicate these infants are more highly exposed in utero to organophosphate pesticides and perform less optimally on an assessment of neurologic integrity at birth than two similar U.S. birth cohorts, the CHAMACOS cohort of California and the Mount Sinai Children's Environmental Health Cohort of New York. The present study is suggestive of a detrimental association between prenatal OP pesticide exposure as measured by urinary DAP metabolite levels and neurobehavioral functioning

at birth, particularly on measures of attention, motor function, and abnormal reflexes. This study represents a much need addition to the current literature by utilizing temporally resolved prenatal exposure data to investigate this association.

Recommendations for Future Research

Additional research is needed to understand how the timing of prenatal exposure to OP pesticides influences specific aspects of neurodevelopment at birth and during childhood. Previous studies have been unable to investigate the potential consequences of trimester specific exposure due to a limited number of biological samples collected during pregnancy, and the present study was unable to fully investigate the impact of first trimester exposure due to a limited number of participants enrolled early enough. Future studies should be designed to incorporate an adequate quantity of biomarker measures in order to properly assess in utero exposure.

In addition, further research is needed to determine whether early markers of impaired neurologic integrity as measured by the BNBAS persist over time, and whether this assessment is predictive of future measures of neurodevelopment.

Policy Recommendations

While prenatal exposure to neurotoxic pesticides is a worldwide public health issue, this problem is especially concerning in developing countries, including Thailand, where regulations are absent or unenforced, and where

capacity to evaluate the health effects is limited. In the United States we are fortunate to have the capacity to research this issue, and many studies have provided sound scientific evidence linking low-level exposure to OP pesticides to a range of adverse health outcomes, including neurodevelopment (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). Unfortunately, our current regulatory system has failed to heed these warnings. Current risk assessment standards are based on less sensitive endpoints and do not adequately protect infants and children from adverse neurodevelopmental effects (Rauh et al., 2006; Rauh et al., 2012). One of the gaps preventing the translation of this research into policy was the absence of time-resolved pregnancy exposure data. Our hope is that this pilot study and our planned future studies in Thailand will provide the final impetus for regulatory reform for OP pesticides to be more protective for children's environmental health.

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VII. TABLES AND FIGURES

Table 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

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%
Maternal 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		
1,500 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)	13	23.2%
Second (12 - 24 weeks)	43	76.8%
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%

*6,000 Baht ~ 200USD; Incomes below 6000 baht/month are considered below the poverty level

Table 2. continued

	n			*%*
Infant Sex				
Male	28			50.0%
Maternal Pre-pregnancy BMI				
Underweight	7			12.7%
Normal	44			80.0%
Overweight	3			5.5%
Obese	1			1.8%
	n	*Mean*	*SD*	*Range*
Maternal Age	56	26.3	4.7	18.0 - 35.0
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
Maternal Pre-Pregnancy BMI	56	20.9	2.6	16.4 - 30.2
Birth weight	56	2,862.5	420.3	1,560.0 - 3,750.0
Gestational Age at Delivery	56	38.7	1.5	35.0 - 42.7
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 Dialkylphosphate Metabolite Levels (nmol/L)

Biomarker of Exposure	*n*	*Mean*	*Median*	*SD*	*Range*	*IQR*
Total Pregnancy DAPs	56	365.0	187.8	383.93	42.4 - 1,759.4	94.1 - 490.8
DEPs	56	328.2	152.9	381.2	20.8 - 1,736.5	73.3 - 459.7
DMPs	56	38.1	24.6	48.2	16.6 - 320.6	17.6 - 32.1
First Trimester DAPs	13	463.6	234.8	851.0	27.5 - 3,170.9	63.2 - 361.2
DEPs	12	405.9	151.9	875.6	10.3 - 3,154.0	38.3 - 298.6
DMPs	13	83.1	17.3	143.5	16.9 - 525.2	17.3 - 90.6
Second Trimester DAPs	56	458.1	209.0	579.5	30.3 - 2,897.6	108.3 - 713.3
DEPs	56	407.4	175.9	568.7	13.1 - 2,866.4	87.9 - 487.8
DMPs	56	50.7	18.8	110.3	15.7 - 724.9	17.3 - 33.4
Third Trimester DAPs	56	298.6	128.0	344.1	26.7 - 1,393.1	59.5 - 450.0
DEPs	56	271.2	103.3	338.9	9.4 - 1,375.8	43.5 - 426.1
DMPs	56	29.2	21.2	23.6	17.3 - 171.2	17.3 - 32.8

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 - 44.0	63
Motor	55	25.3	26.0	4.2	11.0 - 35.0	24.0 - 28.0	45
Range of State	55	13.8	14.0	1.7	11.0 - 17.0	13.0 - 15.0	36
Regulation of State	55	16.4	16.0	2.7	13.0 - 26.0	15.0 - 18.0	36
Autonomic Stability	53	19.5	20.0	1.6	15.0 - 21.0	19.0 - 21.0	27
Abnormal Reflexes	55	1.15	1.00	1.25	0 .00 - 6.00	0 .00 - 1.00	N/A

Table 5. BNBAS Average Cluster Scores for Study Sample

	n	*Mean*	*Median*	*SD*	*Range*	*% Below Median*
Habituation	55	6.6	6.8	0.7	3.3 - 7.8	45.5%
Orientation	55	5.4	5.6	1.1	2.0 - 7.3	47.3%
Motor	55	4.9	5.0	0.7	2.2 - 6.4	38.2%
Range of State	55	3.9	4.0	0.5	3.0 - 5.5	47.3%
Regulation of State	55	4.1	4.0	0.7	3.3 - 6.5	49.1%
Autonomic Stability	54	6.5	6.5	0.5	5.0 - 7.0	50.0%
Abnormal Reflexes	55	1.2	1.0	1.3	0 .0 - 6.0	23.6%*

*Percent ≥ 2 abnormal reflexes

Table 6. Association between total pregnancy average DAP metabolites and summed scores for six BNBAS clusters

BNBAS Cluster	Total Pregnancy ΣDAP			Total Pregnancy ΣDEP			Total Pregnancy ΣDMP		
	N	*β*	*95% CI*	*N*	*β*	*95% CI*	*N*	*β*	*95% CI*
Habituation [a]	51	-0.97	-2.88, 0.95	51	-0.64	-2.34, 1.07	51	-1.16	-4.18, 1.86
Orientation [b]	55	-5.10	-9.53, -0.68*	55	-3.93	-7.86, 0.01	52	-3.26	-10.93, 4.41
Motor [c]	54	-2.92	-5.65, -0.19*	54	-2.46	-4.87, -0.047*	51	1.20	-3.56, 5.96
Range of State[d]	51	0.25	-0.89, 1.39	51	0.19	-0.82, 1.21	51	0.45	-1.34, 2.24
Regulation of State [e]	51	0.77	-0.90, 2.45	51	0.32	-1.18, 1.82	55	1.65	-1.11, 4.41
Autonomic Stability [f]	49	-0.30	-1.45, 0.85	49	-0.16	-1.16, 0.84	49	-0.26	-1.98, 1.45

[a] ΣDAP and ΣDEP adjusted for maternal education, income & pre-pregnancy BMI; ΣDMP adjusted for income & pre-pregnancy BMI
[b] ΣDMP adjusted for maternal age, maternal education, and income
[c] ΣDAP and ΣDEP adjusted for pre-pregnancy BMI; ΣDMP adjusted for pre-pregnancy BMI and income
[d] ΣDAP and ΣDEP adjusted for maternal age, maternal education, income & pre-pregnancy BMI; ΣDMP adjusted for maternal education, income & pre-pregnancy BMI
[e] ΣDAP and ΣDEP adjusted for maternal education, income, and pre-pregnancy BMI
[f] ΣDAP adjusted for maternal age, maternal education, income, and pre-pregnancy BMI; ΣDEP adjusted for maternal education, income & pre-pregnancy BMI; ΣDMP adjusted for income and pre-pregnancy BMI
*p<0.05

Table 7. Association between first trimester average DAP metabolites and summed scores for six BNBAS clusters

BNBAS Cluster	First Trimester ΣDAP			First Trimester ΣDEP			First Trimester ΣDMP		
	N	*β*	*95% CI*	*N*	*β*	*95% CI*	*N*	*β*	*95% CI*
Habituation [a]	13	-0.90	-3.62, 1.83	12	-0.78	-0.367, 2.12	13	1.32	-2.60, 5.24
Orientation [b]	13	-1.98	-8.17, 4.22	12	-2.01	-7.71, 3.70	13	-1.53	-8.99, 5.93
Motor [c]	13	-0.26	-5.82, 5.31	12	-0.97	-5.41, 3.47	13	3.08	-2.84, 8.99
Range of State[d]	13	-0.29	-1.92, 1.35	12	-0.30	-1.99, 1.39	13	0.65	-1.1, 2.39
Regulation of State [e]	13	0.36	-4.18, 4.89	12	0.37	-3.9, 4.64	13	-1.68	-6.83, 3.47
Autonomic Stability [f]	13	-0.20	-1.86, 1.45	12	-0.19	-1.85, 1.47	13	0.55	-1.45, 2.54

[a] ΣDEP adjusted for maternal education and pre-pregnancy BMI; ΣDMP adjusted for maternal age and maternal education

[b] ΣDAP adjusted for maternal age, maternal education, income & pre-pregnancy BMI; ΣDEP adjusted for maternal education, income & pre-pregnancy BMI; ΣDMP adjusted for maternal age, maternal education & income

[c] ΣDAP adjusted for maternal age and pre-pregnancy BMI; ΣDEP adjusted for pre-pregnancy BMI; ΣDMP adjusted for maternal education

[d] ΣDAP and ΣDEP adjusted for maternal age, maternal education, income & pre-pregnancy BMI; ΣDMP adjusted for maternal education & income

[e] ΣDAP and ΣDEP adjusted for maternal age, maternal education, income & pre-pregnancy BMI; ΣDMP adjusted for maternal age, maternal education & income

[f] ΣDAP and ΣDEP adjusted for maternal age, maternal education, income & pre-pregnancy BMI; ΣDMP adjusted for maternal age, maternal education & income

Table 8. Association between second trimester average DAP metabolites and summed scores for six BNBAS clusters

BNBAS Cluster	Second Trimester ΣDAP			Second Trimester ΣDEP			Second Trimester ΣDMP		
	N	*β*	*95% CI*	*N*	*β*	*95% CI*	*N*	*β*	*95% CI*
Habituation [a]	51	-0.88	-2.56, 0.81	51	-0.57	-2.10, 0.97	55	-1.48	-3.59, 0.62
Orientation [b]	54	-5.69	-9.69, -1.69*	54	-4.66	-8.31, -1.01*	54	-1.28	-6.99, 4.44
Motor [c]	54	-3.49	-5.78, -1.20*	54	-3.06	-5.13, -0.99*	51	-0.09	-3.73, 3.56
Range of State[d]	52	-0.39	-1.31, 0.53	52	-0.39	-1.23, 0.44	54	-0.03	-1.34, 1.29
Regulation of State [e]	51	0.43	-1.06, 1.91	51	0.04	-1.31, 1.39	55	1.38	-0.70, 3.47
Autonomic Stability [f]	49	-0.31	-1.31, 0.68	49	-0.12	-1.03, 0.79	49	-0.65	-1.92, 0.63

[a] ΣDAP and ΣDEP adjusted for maternal education, income & pre-pregnancy BMI

[b] ΣDAP and ΣDEP adjusted for pre-pregnancy BMI; ΣDMP adjusted for maternal age and pre-pregnancy BMI

[c] ΣDAP and ΣDEP adjusted for pre-pregnancy BMI; ΣDMP adjusted for maternal age, maternal education, income & pre-pregnancy BMI

[d] ΣDAP adjusted for income; ΣDEP adjusted for maternal age & income; ΣDMP adjusted for maternal age

[e] ΣDAP and ΣDEP adjusted for maternal education, income, and pre-pregnancy BMI

[f] ΣDAP and ΣDEP adjusted for maternal education, income & pre-pregnancy BMI; ΣDMP adjusted for income & pre-pregnancy BMI

*p<0.05

Table 9. Association between third trimester average DAP metabolites and summed scores for six BNBAS clusters

BNBAS Cluster	Third Trimester ΣDAP			Third Trimester ΣDEP			Third Trimester ΣDMP		
	N	*β*	*95% CI*	*N*	*β*	*95% CI*	*N*	*β*	*95% CI*
Habituation [a]	51	-0.95	-2.60, 0.70	54	-0.73	-2.03, 0.58	54	-1.01	-4.74, 2.73
Orientation [b]	55	-1.49	-5.41, 2.44	55	-1.40	-4.66, 1.85	52	3.16	-6.79, 13.10
Motor [c]	51	-0.49	-3.10, 2.13	51	-0.51	-2.67, 1.66	54	1.97	-3.83, 7.76
Range of State[d]	51	0.33	-0.64, 1.30	51	0.33	-0.47, 1.13	51	0.10	-2.22, 2.42
Regulation of State [e]	51	0.39	-1.07, 1.85	51	0.41	-0.79, 1.62	52	-1.66	-4.97, 1.65
Autonomic Stability [f]	49	-0.26	-1.23, 0.72	49	-0.35	-1.14, 0.44	53	1.40	-0.67, 3.47

[a] ΣDAP adjusted for maternal education, income & pre-pregnancy BMI; ΣDEP adjusted for maternal education & pre-pregnancy BMI; ΣDMP adjusted for maternal age, maternal education & pre-pregnancy BMI

[b] ΣDAP and ΣDEP adjusted for maternal age & maternal education; ΣDMP adjusted for maternal age, maternal education & income

[c] ΣDAP and ΣDEP adjusted for maternal education, income & pre-pregnancy BMI; ΣDMP adjusted for maternal education & pre-pregnancy BMI

[d] ΣDAP and ΣDEP adjusted for maternal education, income & pre-pregnancy BMI; ΣDMP adjusted for maternal age, maternal education, income & pre-pregnancy BMI

[e] ΣDAP and ΣDEP adjusted for maternal education, income, and pre-pregnancy BMI; ΣDMP adjusted for maternal education & income

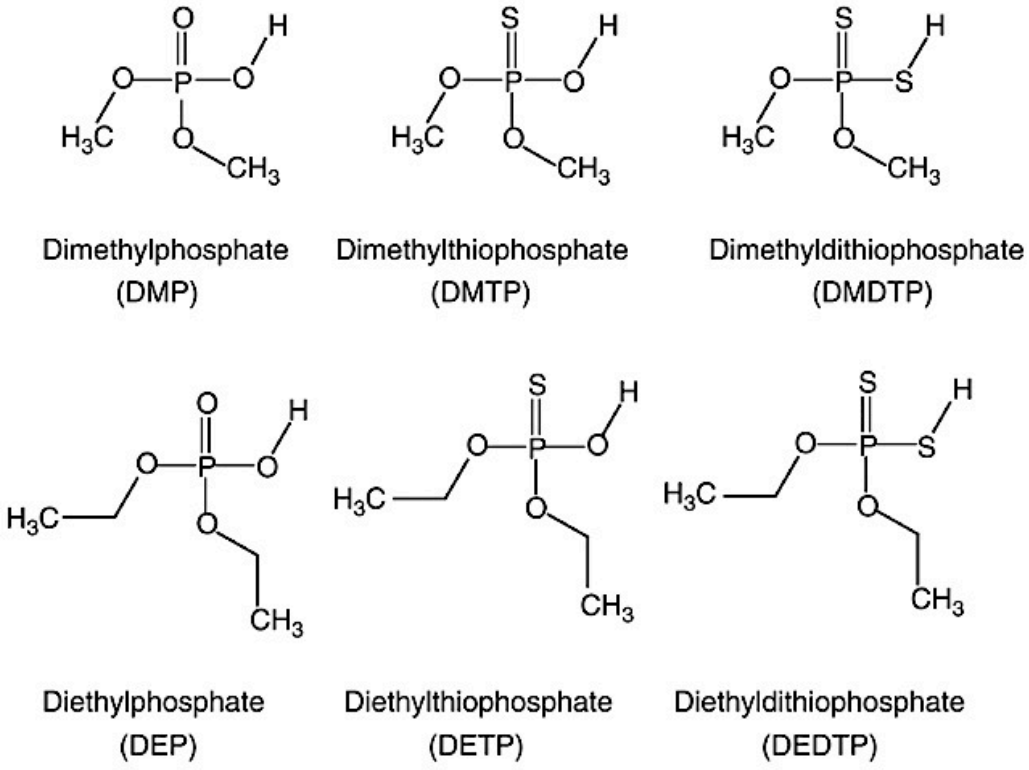
[f] ΣDAP adjusted for maternal age, maternal education, income & pre-pregnancy BMI; ΣDEP adjusted for maternal education, income & pre-pregnancy BMI

Table 10. Association between average DAP metabolites and abnormal reflexes

	ΣDAP			ΣDEP			ΣDMP		
	N	*β*	*95% CI*	*N*	*β*	*95% CI*	*N*	*β*	*95% CI*
Total Pregnancy [a]	54	0.05	-0.66, 0.76	54	-0.001	-0.63, 0.63	54	0.30	-0.74, 1.34
First Trimester [b]	13	0.91	0.29, 1.61*	12	0.57	-0.14, 1.27	13	0.99	0.26, 1.71*
Second Trimester	54	0.17	-0.44, 0.78	54	0.13	-0.41, 0.68	54	0.02	-0.82, 0.87
Third Trimester [c]	54	-0.39	-1.02, 0.24	54	-0.34	-0.84, 0.16	54	-0.12	-1.58, 1.34

[a] ΣDAP adjusted for maternal age; ΣDMP adjusted for pre-pregnancy BMI
[b] ΣDAP adjusted for maternal education; ΣDMP adjusted for maternal education & pre-pregnancy BMI
[c] ΣDAP adjusted for maternal age
*p<0.05

Figure 1. Six common dialkyl phosphate metabolites



Source: Bravo, R. Caltabiano, L.M., Weerasekera, G., Whitehead, R.D., Fernandez, C., Needham, L.L.,…Barr, D.B. (2004). Measurement of dialkyl phosphate metabolites of organophosphorous pesticides in human urine using lyophilization with gas chromatography-tandem mass spectrometry and isotope dilution quantification. *J Expo Anal Environ Epidemiol, 14*(3), 249-259.

Figure 2. Most OP pesticides metabolize to diethyl (A) or dimethyl (B) alkyl phosphates

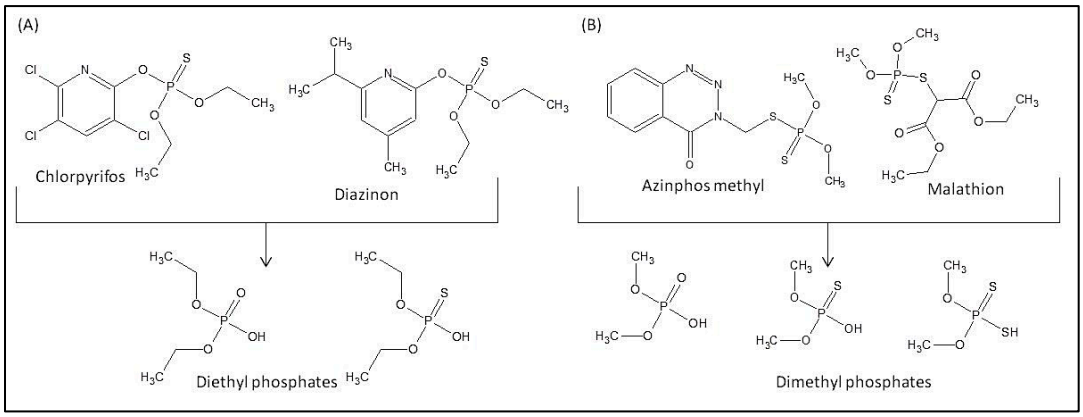


Figure 3. Summary measures obtained for each sample

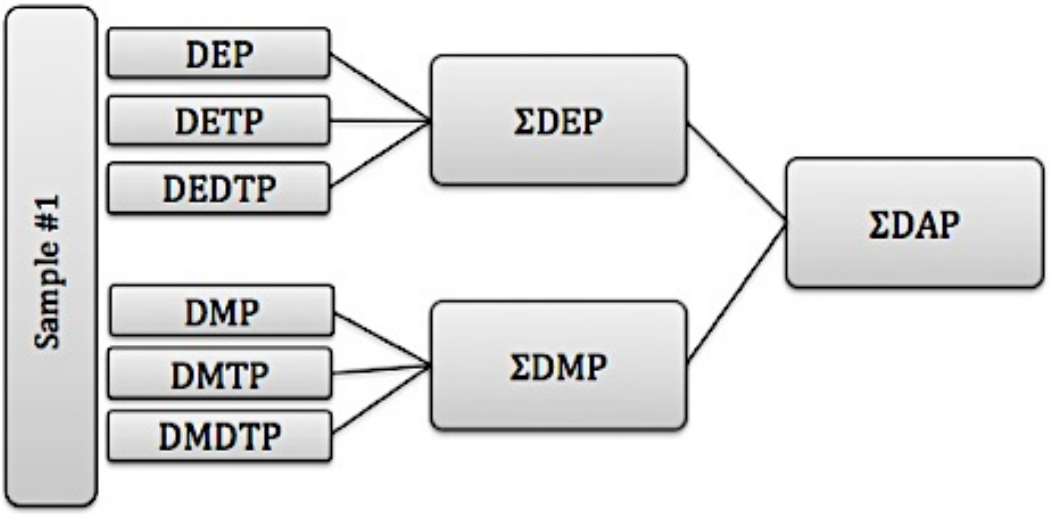


Figure 4. Median concentrations of ΣDAP metabolites in the SAWASDEE cohort

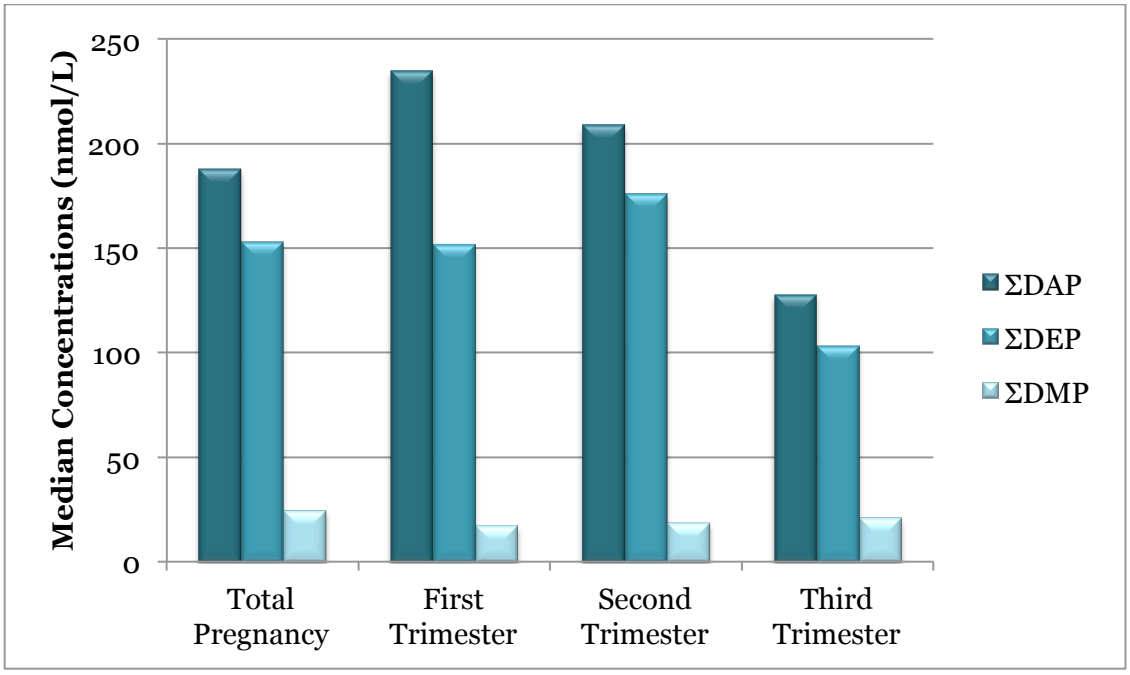


Figure 5. Comparing mean ΣDAP concentrations between the SAWASDEE, CHAMACOS and Mount Sinai cohorts

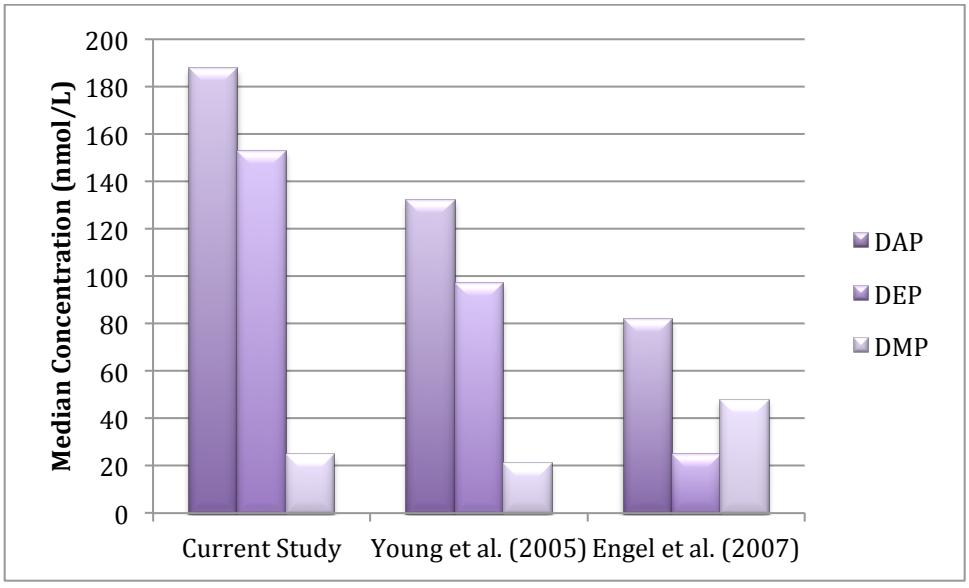
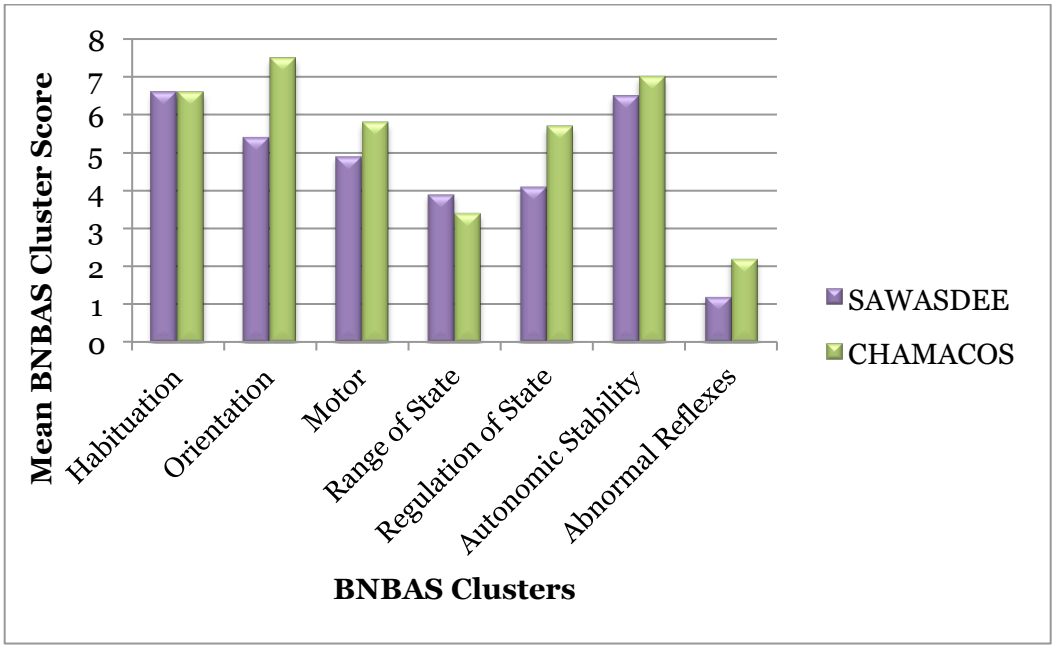


Figure 6. Comparing BNBAS cluster score means between the SAWASDEE and CHAMACOS cohorts



VIII. APPENDICES

A. 1. BNBAS Items

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	

Source: Brazelton, T.B. & Nugent, J.K. (Eds.). (2011) The Neonatal Behavioral Assessment Scale (4th ed.). London: Mac Keith Press.

A. 2. BNBAS Scoring Form

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:

166

Source: Brazelton, T.B. & Nugent, J.K. (Eds.). (2011) The Neonatal Behavioral Assessment Scale (4th ed.). London: Mac Keith Press.

A. 3. Seven Cluster Scoring Method

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

Source: Brazelton, T.B. & Nugent, J.K. (Eds.). (2011) The Neonatal Behavioral Assessment Scale (4th ed.). London: Mac Keith Press.

B. Logistic Regression Analysis

Table 1. Association between total pregnancy average DAP metabolites and average scores for seven BNBAS clusters

BNBAS Cluster	Total Pregnancy ΣDAP			Total Pregnancy ΣDEP			Total Pregnancy ΣDMP		
	N	*OR*	*95% CI*	*N*	*OR*	*95% CI*	*N*	*OR*	*95% CI*
Habituation[a]	54	1.18	0.32, 4.24	54	1.06	0.34, 3.29	54	0.85	0.11, 6.46
Orientation[b]	51	3.75	0.90, 15.60	51	2.82	0.81, 9.81	52	2.38	0.28, 20.11
Motor[c]	51	1.12	0.28, 4.48	51	1.24	0.36, 4.26	51	0.03	<0.001, 1.55
Range of State[d]	51	0.83	0.22, 3.09	51	0.91	0.28, 2.90	51	0.86	0.10, 7.17
Regulation of State[e]	51	0.84	0.20, 3.45	52	0.95	0.29, 3.17	51	0.83	0.08, 8.90
Autonomic Stability[f]	50	1.00	0.25, 3.96	50	0.99	0.30, 3.32	50	0.95	0.11, 7.83
Abnormal Reflexes[g]	54	1.62	0.36, 7.38	54	1.30	0.34, 4.96	54	7.57	0.79, 72.38

[a] ΣDAP, ΣDEP and ΣDMP adjusted for pre-pregnancy BMI
[b] ΣDAP and ΣDEP adjusted for income & pre-pregnancy BMI; ΣDMP adjusted for maternal age & income
[c] ΣDAP and ΣDEP adjusted for income & pre-pregnancy BMI; ΣDMP adjusted for maternal age, income & pre-pregnancy BMI
[d] ΣDAP, ΣDEP & ΣDMP adjusted for income & pre-pregnancy BMI
[e] ΣDAP and ΣDMP adjusted for maternal education, income, and pre-pregnancy BMI; ΣDEP adjusted for maternal education & income
[f] ΣDAP, ΣDEP and ΣDMP adjusted for income & pre-pregnancy BMI
[g] ΣDAP, ΣDEP and ΣDMP adjusted for pre-pregnancy BMI

Table 2. Association between first trimester average DAP metabolites and average scores for seven BNBAS clusters

BNBAS Cluster	First Trimester ΣDAP			First Trimester ΣDEP			First Trimester ΣDMP		
	N	*OR*	*95% CI*	*N*	*OR*	*95% CI*	*N*	*OR*	*95% CI*
Habituation[a]	13	1.79	0.25, 12.68	12	1.87	0.24, 14.28	13	0.93	0.05, 18.14
Orientation[b]	13	19.61	0.13, >999	12	13.72	0.20, 958.57	13	8.46	0.04, >999
Motor[c]	13	3.67	0.36, 37.05	12	4.30	0.41, 44.92	13	0.97	0.04, 23.81
Range of State[d]	13	0.14	0.01, 2.82	12	0.11	0.01, 2.51	13	7.90	0.11, 586.34
Regulation of State[e]	13	0.13	0.01, 3.36	12	0.20	0.01, 3.28	13	0.40	0.01, 29.68
Autonomic Stability[f]	13	1.73	0.03, 113.71	12	1.31	0.20, 8.58	13	0.92	0.04, 20.07
Abnormal Reflexes[g]	13	181.88	0.02, >999	12	11.72	0.21, 669.47	13	203.06	0.49, >999

[a] ΣDEP adjusted for income; ΣDMP adjusted for maternal age, maternal education & income

[b] ΣDAP adjusted for maternal age & pre-pregnancy BMI; ΣDEP adjusted for pre-pregnancy BMI; ΣDMP adjusted for maternal age, maternal education & pre-pregnancy BMI

[c] ΣDAP & ΣDEP adjusted for maternal age, maternal education & pre-pregnancy BMI; ΣDMP adjusted for maternal age, maternal education & income

[d] ΣDAP & ΣDEP adjusted for maternal education & pre-pregnancy BMI; ΣDMP adjusted for maternal age & maternal education

[e] ΣDAP and ΣDEP adjusted for maternal education; ΣDMP adjusted for maternal age & maternal education

[f] ΣDAP adjusted for maternal age & pre-pregnancy BMI; ΣDEP & ΣDMP adjusted for maternal education

[g] ΣDAP & ΣDEP adjusted for maternal age; ΣDMP adjusted for maternal education

Table 3. Association between second trimester average DAP metabolites and average scores for seven BNBAS clusters

BNBAS Cluster	Second Trimester ΣDAP			Second Trimester ΣDEP			Second Trimester ΣDMP		
	N	*OR*	*95% CI*	*N*	*OR*	*95% CI*	*N*	*OR*	*95% CI*
Habituation[a]	54	1.43	0.46, 4.43	54	1.25	0.45, 3.46	55	0.87	0.19, 4.01
Orientation[b]	54	3.72	1.07, 12.88*	54	3.10	1.00, 9.56	52	1.63	0.33, 8.04
Motor[c]	51	1.63	0.47, 5.66	51	1.77	0.57, 5.49	51	0.07	0.002, 1.83
Range of State[d]	52	1.68	0.55, 5.19	52	1.74	0.63, 4.81	55	1.18	0.26, 5.36
Regulation of State[e]	52	2.26	0.68, 7.57	52	1.92	0.65, 5.69	52	2.26	0.37, 13.65
Autonomic Stability[f]	53	1.03	0.32, 3.32	53	0.90	0.31, 2.60	51	1.11	0.23, 5.33
Abnormal Reflexes[g]	54	2.20	0.57, 8.44	54	1.87	0.55, 6.37	54	3.18	0.62, 16.25

[a] ΣDAP & ΣDEP adjusted for pre-pregnancy BMI
[b] ΣDAP and ΣDEP adjusted for pre-pregnancy BMI; ΣDMP adjusted for maternal age & income
[c] ΣDAP and ΣDEP adjusted for income & pre-pregnancy BMI; ΣDMP adjusted for maternal age, income & pre-pregnancy BMI
[d] ΣDAP and ΣDEP adjusted for income
[e] ΣDAP adjusted for income; ΣDEP & ΣDMP adjusted for maternal education & income
[f] ΣDAP & ΣDEP adjusted for pre-pregnancy BMI; ΣDMP adjusted for income
[g] ΣDAP, ΣDEP & ΣDMP adjusted for pre-pregnancy BMI
*p<0.05

Table 4. Association between third trimester average DAP metabolites and average scores for seven BNBAS clusters

BNBAS Cluster	Third Trimester ΣDAP			Third Trimester ΣDEP			Third Trimester ΣDMP		
	N	*OR*	*95% CI*	*N*	*OR*	*95% CI*	*N*	*OR*	*95% CI*
Habituation[a]	55	1.17	0.40, 3.42	55	1.20	0.49, 2.93	51	0.20	0.01, 3.32
Orientation[b]	52	2.13	0.68, 6.67	52	1.89	0.73, 4.88	52	0.58	0.03, 9.88
Motor[c]	54	1.01	0.32, 3.18	54	1.09	0.42, 2.80	51	0.06	0.001, 2.57
Range of State[d]	55	0.79	0.27, 2.31	55	0.79	0.32, 1.93	51	1.52	0.11, 21.10
Regulation of State[e]	52	0.61	0.18, 2.03	52	0.66	0.24, 1.79	54	0.74	0.05, 11.01
Autonomic Stability[f]	51	1.11	0.35, 3.47	51	1.35	0.53, 3.50	53	0.12	0.01, 2.20
Abnormal Reflexes[g]	54	0.82	0.22, 3.08	54	0.77	0.26, 2.33	54	4.22	0.25, 72.10

[a] ΣDMP adjusted for income & pre-pregnancy BMI
[b] ΣDAP and ΣDEP adjusted for income; ΣDMP adjusted for maternal age, maternal education & income
[c] ΣDAP and ΣDEP adjusted for pre-pregnancy BMI; ΣDMP adjusted for maternal age, income & pre-pregnancy BMI
[d] ΣDMP adjusted for income & pre-pregnancy BMI
[e] ΣDAP and ΣDEP adjusted for maternal education & income; ΣDMP adjusted for maternal education & pre-pregnancy BMI
[f] ΣDAP & ΣDEP adjusted for income; ΣDMP adjusted for pre-pregnancy BMI
[g] ΣDAP, ΣDEP & ΣDMP adjusted for pre-pregnancy BMI

C. IRB Approval

EMORY
ROLLINS
SCHOOL OF
PUBLIC
HEALTH

Department of Environmental Health

22 October 2013

RE: IRB approval for Emilia Matthews' Thesis Project

To whom it may concern:

Having previously served on CDC's Institutional Review Board (IRB) of human subjects research evaluation, I am well versed in the Code of Federal Regulations Title 45 Public Welfare, Part 46 Protection of Human Subjects. According to the definition of "research" and the public health practice exempt from IRB approval as designated in 45 CFR 46.102 (b4) and 45 CFR 46.101 (2), respectively, the thesis project proposed by MPH candidate Emilia Matthews and titled "Prenatal Organophosphate Pesticide Exposure and Neurodevelopmental Outcomes in a Thai Agricultural Birth Cohort" is not considered to be research on human subjects and is therefore exempt from IRB approval. Although the study itself has IRB approval (IRB00018962), the subject recruitment/sample collection phase is completed and only data analysis remains. Ms. Matthews will only be working with extant data and will have no interaction with subjects and no subject identifiers.

Sincerely,

Dana B. Barr

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