Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____	_____
Jamie E. Schenk	Date

Prenatal Heavy Metal Exposure and Neurodevelopmental Outcomes in a Thai Agricultural Birth Cohort

By

Jamie Erica Schenk
Master of Public Health

Environmental Health

_____

Dana Boyd Barr, Ph.D.
Committee Chair

_____

Paige Tolbert, Ph.D.
Committee Member

Prenatal Heavy Metal Exposure and Neurodevelopmental Outcomes in a Thai Agricultural Birth Cohort

By

Jamie Erica Schenk

B.S.
University of California, Los Angeles
2013

Thesis Committee Chair: Dana Boyd Barr, Ph.D.

A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Environmental Health
2015

Abstract

Prenatal Heavy Metal Exposure and Neurodevelopmental Outcomes in a Thai Agricultural Birth Cohort

By Jamie Schenk

Current research indicates that low-level prenatal exposure to heavy metals can have increasing neurotoxic effects, especially when exposure is during critical windows of development. Heavy metals can enter the environment through the use of synthetic products, such as pesticides, soldering materials, and paints. Heavy metals can also occur naturally, but rarely at toxic levels or with little bioavailability. The present study aimed to assess the relationship between in utero heavy metal exposure from maternal pesticide application and neurologic integrity at birth. Neurobehavioral function was measured using the Brazelton Neonatal Behavioral Assessment Scale (BNBAS), which utilizes seven clusters (Habituation, Orientation, Motor, Range of State, Regulation of State, Autonomic Stability, and Abnormal Reflex). Trimester-resolved concentrations of five heavy metals (chromium, arsenic, cadmium, mercury, and lead) were measured in blood to assess exposure to the fetuses of tangerine farmworkers in Northern Thailand. These farmworkers are participating in a pilot birth cohort called 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 demonstrate that these infants are more highly exposed in utero to heavy metals and perform less optimally on the BNBAS. Significant associations were seen between arsenic levels and diminished Orientation (β=-1.06, 95% CI: -1.65, -0.48) and Motor (β=-1.13, 95% CI: -1.61, -0.64) clusters of BNBAS. For the Abnormal Reflexes cluster of BNBAS, it was demonstrated that there is a significant association between increased heavy metal levels and increased abnormal reflexes for each metal analyzed. The greatest significances were seen in arsenic in enrollment samples (β=1.06, 95% CI: 0.60, 0.85), cadmium in second timepoint samples (β=1.60, 95% CI: 0.54, 4.75), cadmium in third timepoint samples Cd: β=1.32, 95% CI: 0.56, 3.10), and arsenic in cord blood samples (β=1.07, 95% CI: 0.90, 1.28). Ultimately, these results are suggestive of a negative association between prenatal heavy metal exposure and neurobehavioral functioning at birth. This study is one of the first to examine the impact of trimester-specific exposure to heavy metals on neurodevelopment using several measures of exposure biomarkers in a highly exposed agricultural population.

Acknowledgements

From Emory University, I would like to thank Drs. Dana Barr and P. Barry Ryan, the PI of the study, Parinya Panuwet, Anne Reiderer, Jordan Cohen, Emilia Matthews, Grant Walter, and the support staff and graduate research assistants of the Analytical Exposure Science and Environmental Health Laboratory. From Chiang Mai University (CMU), I would like to thank Tippawan Prapamontol, Warangkana Narksen, and other CMU staff and team members.

I would also like to extend a special thank you to my family and friends for their constant encouragement, love, and support throughout the pursuit of my higher education and my thesis.

Table of Contents

Prenatal Heavy Metal Exposure and Neurodevelopmental Outcomes in a Thai Agricultural Birth Cohort

I. BACKGROUND & SIGNIFICANCE

Heavy Metals

Heavy metals are a group of dense metals or their related compounds that are usually associated with environmental pollution or toxicity. They are also classified by their specific gravity that is at least five times the specific gravity of water (5 g/cm^3). Within the last 20 years, the term "heavy metal" has been increasingly used in the literature (D. Caserta 2013). Heavy metals, including arsenic, cadmium, chromium, mercury, and lead, can enter the environment through the use of synthetic products, such as pesticides, soldering materials, and paints. Heavy metals can also occur naturally, but rarely at toxic levels or with little bioavailability. Once heavy metals are introduced into the environment, they will remain because they do not degrade like organic molecules. The only exception is mercury, which can be transformed by microorganisms and volatilize (USDA 2000).

Excess heavy metal accumulation in soils is toxic to humans and other animals. The majority of heavy metal exposure is chronic because of food chain exposure. Acute heavy metal exposure is also possible and occurs through ingestion or dermal contact, but it is rare (USDA 2000). The severity and health outcomes of toxic heavy metal exposure depends on several factors including the type and species of the element, route of exposure, duration of exposure,

frequency of exposure, and a person's individual susceptibility (Tchounwou 2012).

Heavy metal exposure can result in a variety of pathways, but for the purpose of this cohort, we expect the exposure to primarily derive from occupational use of pesticides or from residential sources.

Arsenic

Studies have demonstrated that arsenic can cross the placenta into the fetus, and it can be detected in the placenta and cord blood. Currently, theories explaining arsenic exposure through the placenta state that arsenic can cross the placenta through either cellular uptake or by active transport by amino acid carriers. Chronic high-level inorganic arsenic exposure in utero is associated with increased incidence of preterm delivery, miscarriage, stillbirths, low birth weight, and infant mortality (EPA 2007). Neurodevelopmental defects can occur from prenatal exposure to arsenic, including neural tube defects, exencephaly, and disturbed neurulation. Prenatal exposures to arsenic have been associated with lower intelligence quotient (IQ) scores in school-aged children and can affect learning, resulting in learning disabilities. Additionally, concurrent prenatal exposure to arsenic with other chemicals has demonstrated increased teratogenicity of the chemicals. For example, concurrent exposure to arsenic, lead, and methylmercury has resulted in fetal malformations that were additive (EPA 2007).

Chromium

Occupational exposure to chromium has been demonstrated to show adverse effects during pregnancy. Chromium (VI) exposure during pregnancy

and childbirth has been demonstrated to lead to complications and spontaneous abortions (ATSDR 2012). Currently, there are not any studies regarding developmental effects in humans or animals after inhalation exposure to chromium or its related compounds (ATSDR 2012).

Cadmium

Cadmium is primarily known as being nephrotoxic but has demonstrated neurotoxic effects. Increased exposure to cadmium in utero can lead to neurobehavioral alterations, including decreased exploratory motor activity and avoidance acquisition (ATSDR 2012). There is not any sufficient evidence demonstrating health benefits to cadmium in the human body (Nyanza 2014). Associations between maternal cadmium during pregnancy and lower visual IQ (VIQ), performance IQ (PIQ) and full scale IQ (FSIQ) have been demonstrated, with childhood cadmium exposure being somewhat less influential than maternal exposure during pregnancy for VIQ. Sex differences in the health effects of exposure have been seen in particular with females with childhood IQ, especially PIQ. In females, head circumference was inversely associated with maternal cadmium exposure (D. Caserta 2013).

Co-exposure with other heavy metals, such as lead, can lead to greater adverse health effects. Studies in children have demonstrated that in utero exposure to cadmium is associated with mental retardation, decreased verbal IQ, lower neuropsychological testing performance, learning disability, poor reading performance, neurophysiological evoked potential differences, and behavioral problems (Ciesielski 2012).

Lead

Lead is able to cross the placenta and can be found in breast milk. It can cross the placenta through passive diffusion. The developing fetus can also be exposed to lead through bone development. Lead can accumulate in the body over time, where it is stored in bones along with calcium. During pregnancy, lead is released from bones as maternal calcium and is used to help form the bones of the developing fetus (ATSDR).

The greatest concern of long-term exposure to lead is neurodevelopment alterations in children following prenatal and/or postnatal exposure. Inhalation is the most common route of exposure among workers in industries where lead is involved (ATSDR). Exposure to lead in utero can affect both the developing fetus and health outcomes manifested during childhood. During pregnancy, lead exposure can lead to reduced growth of the fetus and premature birth. In children, lead can lead to behavioral and learning problems, lower IQ and hyperactivity, slowed growth, hearing problems, and anemia (EPA 2014).

Mercury

Mercury can distribute to a number of organs but primarily concentrates in the brain and kidneys. The role of the placenta as a barrier for mercury is not completely clear. Currently, it is thought cellular uptake of mercury is related to its chemical structure. It has been seen that mercury vapor and methyl mercury can easily pass the placenta using passive transport and amino acid carriers (D. Caserta 2013). There is substantial evidence demonstrating that mercury can cross the placenta and accumulate in fetal tissues (McDermott, Bao et al. 2014). Mercury can be excreted in small amounts in urine, feces, through exhalation, or sweating. However, humans lack an extensive mechanism for mercury excretion,

which leads to accumulation in the body with chronic exposure. A decrease in IQ and changes in behavior in children born to women with high concentrations of mercury has been observed (D. Caserta 2013).

Prenatal Exposure

Concerns have been increasing about the neurotoxic effects of prenatal heavy metal exposure to the fetus, including low-level exposure (Michael Lewis 1992). Low doses of toxic metal have shown to be dangerous (Yu, Yan et al. 2011). The fetus is especially vulnerable to exposures during critical windows of development. Heavy metals can cross the placental and blood-brain barriers, inducing neurotoxicity and liver toxicity, as well as bone accumulation and inhibition to vital enzymes in organisms (Zheng, Zhong et al. 2014).

The placental barrier is not completely impermeable to the passage of heavy metals (D. Caserta 2013). Heavy metals are retained in and transferred via the placenta in different amounts. Cord blood concentrations can vary from maternal blood concentrations, as well as the placenta and the cord tissue. If the concentration is higher in the placenta, it implies that the fetus is not as exposed to a particular heavy metal, but this does not put them out of danger. However, if the concentration of a heavy metal is higher in the cord tissue than in the placenta, it indicates that the fetus is being more highly exposed to that particular heavy metal.

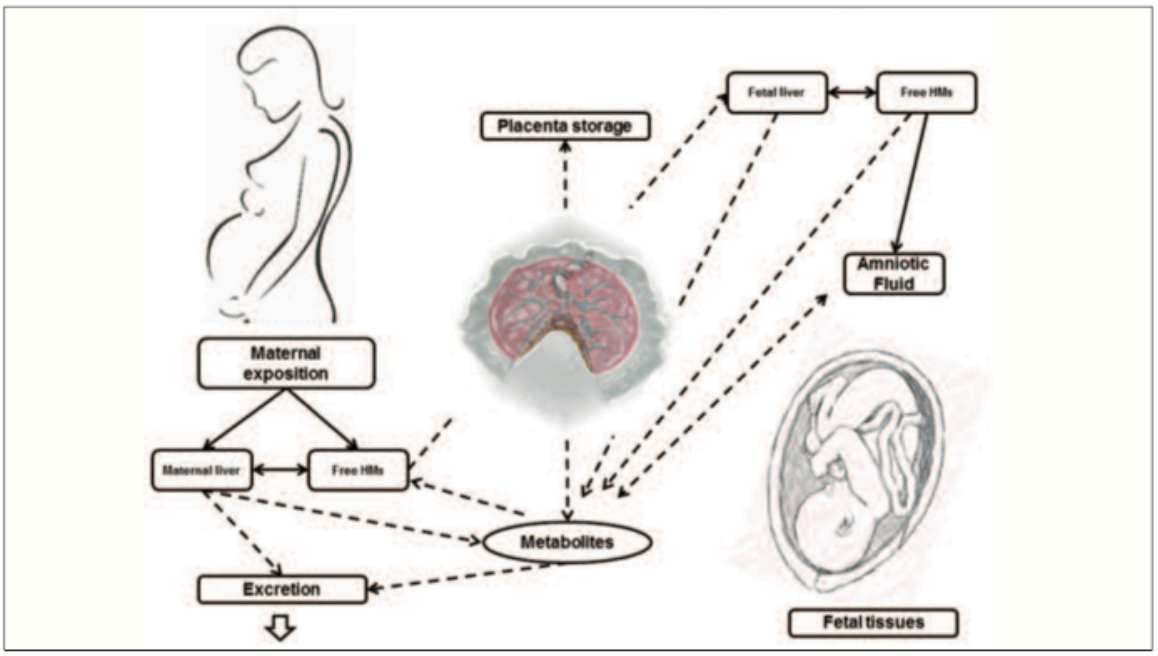

Figure 1: Interactions and passage of heavy metals between maternal circulation and the fetus through the placenta (D. Caserta 2013)

The timing of exposure is critical in determining the effects the exposure will have on the developing fetus. Particularly, being sensitive to organogenesis, or the process where the ectoderm, endoderm, and mesoderm developing into the internal organs, is essential in knowing the health effects that will arise from exposure. If the exposure takes place during organogenesis, heavy metals could produce permanent structural and anatomical changes. Conversely, if the exposure occurs after the end of organogenesis, it might result in functional consequences (Zheng, Zhong et al. 2014).

Sometimes, the presence of one heavy metal may co-occur with the presence of other heavy metals. This makes it difficult to separate their individual contribution to later development (Michael Lewis 1992). Cadmium and lead are commonly found to co-occur. Therefore, adverse outcomes could either be from

the heavy metal's independent impact or their combined impact. It is important to consider the potential confounding of interactions of numerous toxic substances, including heavy metals, during prenatal development (Zheng, Zhong et al. 2014).

Exposure to heavy metals in utero can ultimately result in "behavioral and learning problems, lower IQ and hyperactivity, slowed growth, hearing problems, and anemia" (EPA 2014). Approximately 200,000 children born in Thailand each year are at risk of prenatal exposure to pesticides and associated neurodevelopmental outcomes because of their mothers' agricultural occupational exposures (Lorenz, Prapamontol et al. 2012).

SAWASDEE Birth Cohort

The **S**tudy of **A**sian **W**omen and their off**S**pring's **D**evelopment and **E**nvironmental **E**xposures (SAWASDEE) was conducted in an agricultural region in Northern Thailand. This study examined pesticide biomarker concentrations in pregnant mothers, as well as similar markers in their newborn children. It is the first birth cohort study to have temporally-resolved exposure data, capturing monthly and trimester-specific exposures (Matthews 2014).

The women enrolled in the SAWASDEE pilot birth cohort worked in the agricultural industry as tangerine farmworkers in Fang District, Chiang Mai Province of Northern Thailand. Through their occupation, they were exposed to pesticide residues from their thinning and harvesting activities. Additionally, they may have been exposed to pesticides from residential applications. Dermal exposures from picking or thinning of fruit from the trees shortly after they have

been sprayed with pesticides and inhalation exposure from re-volatilized pesticide residues are the exposures of greatest concern. In the SAWASDEE cohort, exposure levels were measured at multiple time points throughout pregnancy, increasing its strength and validity in estimating exposures that may be linked to neurodevelopmental outcomes.

Studying exposures in female agricultural workers is critical in understanding exposure pathways and health effects. Farming is a large industry worldwide, and the gender division is narrowing. Women are now participating in the same duties as men, such as harvesting crops, pesticide application, and plowing. On average, 43% of the global agricultural workforce is comprised of women (UN 2014). In developing countries in South Asia and Africa, almost 70% of the agricultural workforce is comprised of women. In Fang, the city where the agricultural workers for the SAWASDEE birth cohort reside, 50% of the agricultural workforce is comprised of women.

Pesticide use has increased and developed both domestically and internationally. Within the past 30 years, pesticide use in agriculture and for the home and industrial purposes has increased by 50%. While organophosphate and pyrethroid insecticides are typically considered the most abundant and thus most important neurotoxicant exposures in agriculture, heavy metals are also an important class of neurotoxicants to consider. This is because heavy metals can leach from metal-containing pesticides and are likely also present as environmental contaminants in residential areas. These metal exposures also have potential to induce or increase neurologic deficits in infants exposed prenatally.

Brazelton Neonatal Behavioral Assessment Scale

The Brazelton Neonatal Behavioral Assessment Scale (BNBAS) is a standardized test that assesses neurodevelopment in infants from birth to two months of age (Brazelton 2011). This scale provides a view of the complexity of the neonate, assessing how they handle the transition from an intrauterine, symbiotic condition to a relatively independent experience (Brazelton 1978). The BNBAS also assesses how infants cope with internal and external stimuli. It is a universally accepted assessment scale to evaluate neurodevelopment in early infancy because it does not require any culturally-specific modifications or validation. In this half hour assessment, the neonate exhibits motor, cognitive, social, and temperamental responses, as well as observable psychophysiological reactions to certain testing procedures (Brazelton 1978). The BNBAS is comprised of a set of 18 reflexes and 28 behavioral evaluations to assess seven domains of behavior in infants.

Dialkyl Phosphate Metabolites (DAPs)

A method for analyzing neurodevelopmental outcomes associated with organophosphorus insecticides (OPs) in urine is to measure the dialkyl phosphate metabolites (DAPs), a nontoxic breakdown product. Approximately 75% of OPs are converted into DAPs, which is used to indicate exposure to OPs in urine (CDC 2009). DAPs can be present in urine after low levels of exposure to OPs that do not cause clinical symptoms or inhibition of cholinesterase activity (CDC 2013).

DAPs analysis has been important in studying repeated exposure to OPs, and inherently heavy metals in these insecticides, and neurodevelopmental outcomes (María Teresa Muñoz-Quezada and Rojas 2013). They have been used to study exposure in farmers, agricultural workers, pest-control workers, and others to OPs (CDC 2013). DAPs levels in agricultural workers can either be reflective of the general population's exposure or much higher depending on the season and type of crop application (CDC 2013).

The two DAPs metabolites that have been the most indicative of an association have been the dimethyl and diethylphosphate metabolites. Studies using DAPs analyses and BNBAS tests have demonstrated a significant association with adverse neurodevelopmental outcomes, including smaller head circumference, as well as abnormal reflexes, attention span, orientation, and motor skills (Jessica G. Young 2005, Brenda Eskenazi 2010, Carol J. Burns 2013)

In this study, we are aiming to find similar patterns observed in studies using DAPs and urine but with blood as the matrix of study and heavy metals as the target analytes. We will examine DAPs in our model as potential confounders or effect modifiers.

II. Methods

Participants and Recruitment

The SAWASDEE Study is a longitudinal pilot birth cohort. Its study population consists of farmworker women and neonates residing in the Chiang Mai Province of northern Thailand. Women (N=59) who were in their first or early second trimester of pregnancy were recruited between March 2011 and

February 2012 at the local antenatal clinic at Fang Hospital. 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.

Inclusion criteria in the SAWASDEE Study included:

1) Aged 18-40 years;
2) Thai identification card permitting hospital and antenatal clinic access;
3) Thai as the primary language at home;
4) Residence in their 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.

The possession of a Thai identification card allowed each pregnant woman a minimum of one monthly prenatal visit to an OB/GYN at Fang Hospital. Each participant was followed longitudinally at each prenatal and postnatal visit, until three days after delivery. All women (59/59) who were approached and met the inclusion criteria gave informed consent to be in the study giving a participation rate of 100%. Three participants were lost to follow up or were excluded because of spontaneous abortion. The retention rate was 95%.

Participants were administered a questionnaire at three different times during their participation—at the time of their enrollment, at 28 weeks, and 36 weeks of gestation. This questionnaire collected demographic data including household income, maternal age, maternal education, and maternal occupation,

pesticide use information, and knowledge, attitudes and practices of pesticide use.

Biological samples were collected at each prenatal visit and at parturition. A urine sample was collected at each visit and blood samples were collected at the same time points that the questionnaire was administered. Additional blood and urine (maternal, infant, and cord) were collected at birth.

Maternal blood samples were collected at multiple time points throughout the pregnancy. Two blood samples were collected from the mothers per trimester, as well as cord blood.

Questionnaire and Medical Record Abstraction Data

The questionnaire collected information on 168 multipart questions intended to garner data on maternal and paternal demographics and occupations, housing characteristics and cleanliness, knowledge, attitudes and practices on pesticides use, occupational pesticide exposures, maternal health including history, current health, testing and medication, home pesticide use, household pets, maternal personal habits, and other exposures and concerns.

Medical records were also used to obtain information, including infant sex, birth weight, birth length, gestational age, head circumferences, APGAR scores, and pregnancy or delivery complications.

Exposure Assessment

Heavy metals were measured in 4 whole blood samples for each participant. Briefly, 1 mL blood was spiked with yttrium internal standard, mixed well and digested for 3.5 hours with nitric acid to break down organic molecules.

The digested samples were analyzed for lead, mercury, cadmium, arsenic and chromium using inductively coupled plasma-mass spectrometry. Calibration standards, blanks and quality control samples were analyzed concurrently with unknown samples. All data were integrated and the concentrations calculated using a linear regression line derived from the standard curve. All data were blank-subtracted and had quality control samples that verified method performance.

Outcome Assessment

BNBAS was conducted on each infant within 3 days of birth by a certified administrator of the test. A total of 55 infants were tested using BNBAS, although some infants were missing scores for certain domains. The scoring for BNBAS uses 28 behavioral items and 18 reflexes into seven domains (or clusters), which are: habituation, orientation, motor performance, range of state, regulation of state, autonomic stability, and abnormal reflexes. A nine-point scale was utilized to score each of the 28 behavioral items. Six of the domains are comprised of three to six items. The seventh domain is comprised of the 18 reflex items, which were then scored on a four-point scale indicating the degree of abnormality. The four behavioral domains look to see the following information in the dimensions of newborn organization (Brazelton 1978):

1. Interactive capacities, assessing the newborn's capacity to attend to and process simple and complex environmental events
2. Motor capacities, assessing the infant's capacity to attend to and process simple and complex environmental events

In BNBAS, higher scores represent more optimal functioning. Cluster scores for the six domains concerning the 28 behavioral are calculated by recording the original BNBAS items when necessary. The recorded individual items within each domain are then averaged. An average score for each domain is calculated, forming a summary score for interpretation. For each domain, a normal score varies.

Aims and Hypotheses

Aim I: To determine in utero exposure levels of heavy metals

- **Hypothesis:** Heavy metal concentrations will vary over the course of pregnancy due to peaks in agricultural seasons and pesticide application, which would require multiple measures over exposure during the pregnancy.

Aim II: To determine if the neurodevelopmental outcomes are associated with heavy metal exposure

- **Hypothesis 1a:** Maternal heavy metal levels will be associated with abnormal time sensitive attention parameters as measured by the BNBAS.
- **Hypothesis 1b:** Maternal heavy metal levels will be associated with abnormal reflexes as measured by the BNBAS.

Data Analysis

Data will be analyzed using SAS statistical software, version 9.4 (SAS Institute, Inc., Cary, North Carolina). To address Aim I, univariate analysis will be conducted to describe exposure and outcomes measures and to assess skewness. To address Aim II, linear and logistic regression will be utilized. Linear

regression will be utilized to analyze six of the seven domains tested in BNBAS. Reflexes will be analyzed using logistics regression.

To assess in utero exposures to heavy metals, univariate analyses were conducted to assess exposure levels to the five heavy metals of interest (chromium, arsenic, cadmium, mercury, and lead) in each of the collection timepoints (enrollment, second timepoint, third timepoint, cord blood at birth).

To assess the relation between heavy metal levels and neonatal performance levels on BNBAS, separate linear regression models were fit for six of the seven domains. The seventh domain, abnormal reflexes was analyzed using Poisson regression. This is because the data for abnormal reflexes was derived from counts rather than scores.

Based on previous literature, a number of potential confounders were considered, including gestational age, maternal age, maternal education, household income, and residential factors, including pesticide use, dust, and paint.

III. Results

Demographic Data

Table 2 displays demographic characteristics of SAWASDEE pilot birth cohort participants. The participants were predominately young women (mean age=26.3 years) born in Burma (64.3%) and were of Thai Yai Ethnicity (60.7%). Most participants were unmarried but living as such (91.1%). Participants mainly had low educational attainment, with the majority of participants never having attended school (64.3%). Most participants have low household incomes below

the poverty line, with 66.1% of participants living on ≤6,000 Baht ($184.15 USD) or less per month.

Participants mainly enrolled during their second trimester (76.8%) versus the first trimester (23.2%) of their pregnancy. The season with the greatest enrollment (60.7% was the rainy season (May-October)), when pesticides were not actively applied. The majority of women in the SAWASDEE birth cohort delivered their infants to term (85.7%), and these infants were of normal birth weight (87.5%). The SAWASDEE birth cohort included eight preterm infants, with a gestational age < 37 weeks, and eight low birth weight low birth weight, classified as weights < 2500 g. A total of four infants were both preterm and low birth weight.

Exposure Distribution

Exposure distributions for heavy metal concentrations during the pregnancy are presented in ***Table 3***. Blood samples were collected at enrollment, 28 weeks, and at delivery from the mother, as well as from the umbilical cord at birth. 55 samples were collected at enrollment, and 45 samples of cord blood were collected. 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. For chromium, the mean level was lowest at enrollment (0.6 ng/mL) and the highest at the third timepoint (1.7 ng/mL). The mean level of arsenic was greatest at the third timepoint (2.4 ng/mL) and the lowest in the second timepoint (1.8 ng/mL). For cadmium, the mean level was greatest was at enrollment and the third timepoint (0.8 ng/mL)

and 0.7 ng/mL in cord blood the second timepoint. For mercury, the average concentration was greatest at enrollment (1.4 ng/mL) but was the lowest during the second and third timepoints (1.2 ng/mL at each timepoint). For lead, the mean lead level at enrollment was highest at 29.6 ng/mL and 26.2 ng/mL at the second timepoint.

Regarding cord blood samples, the median concentrations of the heavy metals greatly varied. This is due to each metal's ability to cross the placental barrier and the half-life of each heavy metal. Cadmium had the lowest median concentration (0.1 ng/mL). Chromium had a median concentration of 0.4 ng/mL. Arsenic had a median concentration of 1.3 ng/mL, and mercury had a median concentration of 1.8 ng/mL. The metal with the greatest median concentration in cord blood was lead at 17.5 ng/mL.

Correlations of maternal blood samples for each analyte and cord blood were also examined, with their correlation coefficient and p-values (α=0.05) displayed in ***Tables 4-8***. Correlation analysis of chromium did not yield any statistically significant results. There was significance correlation seen with arsenic levels between enrollment and the second timepoint of collection (r=0.45, p=0.001), as well as between the third timepoint of collection and cord blood (r=0.64, p=<0.0001). Significance correlations were also seen between maternal cadmium samples and cord blood between enrollment and the second timepoint of collection (r=0.90, p=<0.0001), enrollment and the third timepoint of collection (r=0.88, p=<0.0001), and the second timepoint of collection and the third timepoint of collection (r=0.92, p=<0.0001). Associations of statistical significance were seen with maternal mercury blood samples and cord blood

between enrollment and the second timepoint of collection (r=0.52, p<0.0001), enrollment and the third timepoint of collection (r=0.51, p=0.0002), the second timepoint and third timepoint of collection (r=0.45, p=0.002), second timepoint of collection and cord blood (correlation=0.60, p=<0.0001), and third timepoint of collection and cord blood (r=0.38, p=0.01). For lead, each maternal timepoint analysis with cord blood demonstrated a statistically significant association, with all p-values <0.0001.

Tables 9-12 display the correlation coefficients for each of the heavy metals analytes at each timepoint of collection with correlation coefficients and their associated values. At each collection period of blood (maternal, cord), none of the metals demonstrated a statistical significance in their correlation.

Outcome Distribution

Table 13 displays the sample mean and median values, standard deviation, and ranges for the summed measures of each of the seven cluster scores that comprise BNBAS. These cluster scores were used in the linear and logistic 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. Excluding the Abnormal Reflux cluster, the Habituation cluster has the strongest performance by the infants tested. This can be seen with its higher mean (26.5) and median (27.0) scores when considering the highest possible score for the cluster (36). The cluster with the least variation was Autonomic Stability, with a range between 15.0-21.0. Range of State was the cluster with the lowest mean (16.0).

Regarding the Abnormal Refluxes cluster, the average infant had approximately one abnormal reflex. The range for this cluster is between 0.00 and 6.00.

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 enrollment, timepoint, and cord blood averages of the heavy metals measured during and after pregnancy.

For enrollment samples, a significant association was found between cadmium levels and the Abnormal Reflexes cluster of BNBAS with an odds ratio of 0.24 (95% CI: 0.06, 0.96), demonstrating a protective effect of cadmium.

In second timepoint samples, a significant association was seen between arsenic levels and the Motor cluster of BNBAS with an odds ratio of 2.03 (95% CI: 1.06, 3.87), Range of State cluster of BNBAS with an odds ratio of 0.31 (95% CI: 0.15, 0.65), and Regulation of State cluster with an odds ratio of 0.49 (95% CI: 0.26, 0.95).

The third timepoint has shown some statistical significance regarding arsenic levels and the Habituation, Orientation, and Motor clusters of BNBAS. Arsenic levels and the Habituation cluster had an odds ratio of 0.78 (95% CI: 0.65, 0.94), arsenic levels and the Orientation cluster had an odds ratio of 1.31 (95% CI: 1.08, 1.58), and arsenic and Motor cluster had an odds ratio of 1.36 (95% CI: 1.11, 1.66).

For cord blood, a significant association was found between increasing lead levels and the odds of a less-optimal performance on the Habituation cluster of BNBAS with an odds ratio of 0.96 (95% CI: 0.91, 1.01). Significance was also seen in chromium levels and a less-optimal performance on the Range of State cluster of BNBAS with an odds ratio of 2.33 (95% CI: 1.10, 4.98).

Linear Regression Analysis

Tables 14-17 show results of the linear regression models, with regression coefficients and confidence intervals for six of the BNBAS clusters, excluding abnormal reflexes, with enrollment, second timepoint, and third timepoint exposures, as well as cord blood concentration. There was not any significance demonstrated between enrollment average heavy metal analyte levels and the summed scores for six BNBAS clusters.

At the second timepoint, a significant association was seen with arsenic and lead levels and the Habituation cluster (As: β=-1.75, 95% CI: -2.79, -0.07; Pb: β=0.09, 95% CI: 0.01,0.17). Significant associations were also seen with arsenic levels and the Range of State (β=-0.43, 95% CI: -0.43, 1.69) and Autonomic Stability clusters (β= -0.69 95% CI: -1.29, -0.10). These significant associations indicate that with increasing levels of the arsenic and lead, there is a decrease in BNBAS scores.

Significant associations were seen at the third timepoint. With arsenic, there were significant associations with the Orientation (β=-1.06, 95% CI: -1.65, -0.48) and Motor (β=-1.13, 95% CI: -1.61, -0.64) clusters of BNBAS. A significant association was also seen with cadmium levels and the Autonomic Stability

cluster (β=-1.94, 95% CI: -3.26, -0.62). These significant associations indicate that with increasing levels of the arsenic and cadmium, there is a decrease in BNBAS scores.

In cord blood, significant associations were seen in lead levels in the Range of State cluster (β=0.05, 95% CI: 0.004, 0.10) and mercury levels in the Regulation of State cluster (β=0.87, 95% CI: -1.60, -0.13).

Poisson Regression Analysis

Table 18 displays the results of the Poisson regression models, with adjusted regression coefficients and 95% confidence intervals for abnormal reflexes. Associations between enrollment levels of chromium, arsenic, cadmium, mercury, and lead and the Abnormal Reflex cluster were significant (Cr: β=0.99, 95%: 0.60, 1.62; As: β=1.06, 95% CI: 0.60, 0.85; Cd: β=1.05, 95% CI: 1.12, 3.77; Hg: β=0.78, 95% CI: 0.50, 1.21; Pb: β=0.99, 95% CI: 0.97, 1.02).

Between the second timepoint levels of chromium, arsenic, cadmium, mercury, and lead and the Abnormal Reflex cluster, significance was seen with each of the heavy metals measured (Cr: β=0.89, 95%: 0.69, 1.16; As: β=1.07, 95% CI: 0.74, 1.55; Cd: β=1.60, 95% CI: 0.54, 4.75; Hg: β=0.80, 95% CI: 0.42, 1.52; Pb: β=0.99, 95% CI: 0.96, 1.02).

Associations between third timepoint levels of chromium, arsenic, cadmium, mercury, and lead and the Abnormal Reflex cluster were significant Cr: β=1.00, 95%: 0.92, 1.00; As: β=1.09, 95% CI: 1.04, 1.12; Cd: β=1.32, 95% CI: 0.56, 3.10; Hg: β=0.96, 95% CI: 0.61, 1.49; Pb: β=1.00, 95% CI: 0.97, 1.03).

Between the cord blood levels of chromium, arsenic, cadmium, mercury, and lead and the Abnormal Reflex cluster, significance was seen with each of the heavy metals measured (Cr: β=0.66, 95%: 0.30, 1.45; As: β=1.07, 95% CI: 0.90, 1.28; Cd: β=4.22, 95% CI: 0.40, 44.81; Hg: β=0.84, 95% CI: 0.55, 1.27; Pb: β=1.02, 95% CI: 0.96, 1.08). This indicates an increase in abnormal reflexes with increasing chromium, arsenic, cadmium, mercury, and lead levels.

IV. DISCUSSION

Interpretation of Results

To date, few studies have evaluated the neurological impacts from prenatal exposure to heavy metals from high exposure level populations utilizing a birth cohort study design, which is displayed in ***Table 5*** (included in ***Appendix B***). Grandjean et al. studied the Faroese birth cohort utilizing cord blood, maternal hair, and samples from the child of blood and hair at age 7 to assess postnatal methylmercury exposure from seafood and demonstrated that postnatal exposure to methylmercury lead to deficiencies in neurodevelopment (Grandjean P 2014). Concomitant exposure using methylmercury and lead was studied by Yorifuji et al. evaluating the associations between cord blood lead concentrations and cognitive deficiencies. This study supports that there are adverse effects on cognitive functions at elevated concentrations of lead in cord blood, but its combined effects with methylmercury were inconsistent (Yorifuji T 2011). Benchmark dose calculations were made utilizing both the Faroese and Madeiran birth cohorts, which are both from populations with increased exposures to methylmercury from seafood consumption. This study demonstrated that there

were significant delays on evoked potential latencies in both cohorts related to higher levels of methylmercury (Murata K 2002).

A study pooling seven international population-based longitudinal cohort studies from infancy to 5-10 years of age looked at low-level environmental lead exposure and children's intellectual function (full-scale IQ). This study demonstrated that environmental lead exposure in children who have maximal blood lead levels of <7.5 μg/dL.

An Italian birth cohort established in Northeastern Italy near a contaminated site from mercury pollution looked at total mercury and methyl mercury in maternal hair, breast milk, and in the child's hair. Children born to mothers with elevated levels of total mercury had full scale, verbal, and performance IQs that were 4-5 points lower than children born with lower levels of total mercury, but these findings were not statistically significant (Deroma L and F. 2013). Another Italian cohort study was conducted in Northern Italy looking at lead poisoning in newborns and infants, utilizing venous blood specimens collected at three timepoints, demonstrating that lead was present in participants regardless of their residential location (rural versus urban), but the need for a general screening program remains inconclusive for this particular population (Garbo G 1998).

Four prospective birth cohorts from Mexico City were used to assess windows of susceptibility to lead and cognitive effects. Multiple blood lead levels from children 1-4 years old, and children's cognitive abilities at four years of age were assessed. The study demonstrated that higher blood lead concentrations at

two years old were the mot predictive of decreased cognitive abilities among the children of these cohorts (Braun JM, Solano-Gonzalez M et al. 2012).

In the Seychelles, a neurodevelopmental study assessing in utero exposure to methylmercury from maternal fish diet was studied using maternal hair samples during pregnancy. To assess neurodevelopment, the revised Denver Developmental Screening Test (DDST-R) and a neurological examination were utilized, and an association between fetal mercury exposure and development was found (Myers GJ and Cernichiari E 1995).

The aforementioned birth cohorts provide critical knowledge to studying exposures within the SAWASDEE birth cohort and areas to expand research directions. The SAWASDEE birth cohort is unprecedented in its efforts, utilizing both blood and urine samples collected at specific timepoints to capture trimester specific exposure. The present study collected an average of 3 blood samples from the mother and 1 blood sample from the umbilical cord. Therefore, we were able to characterize infants' exposure to heavy metals over the course of pregnancy more accurately than past studies. Additionally, we were able to independently assess the effect of trimester specific exposure on each of the BNBAS outcomes. This study adds to the growing body of evidence that suggests that prenatal heavy metal exposures, even in the presence of acute neurotoxic pesticide exposures, can adversely alter neurodevelopmental trajectories.

The results of analyses based on the SAWASDEE pilot birth cohort of 56 neonates born to Thai agricultural workers are suggestive of adverse neurodevelopmental effects of in utero heavy metal exposures from pesticide residues. The heavy metals measured included chromium, arsenic, cadmium,

mercury, and lead. Particularly, these adverse effects were seen in the Habituation, Motor, Range of State, and Abnormal Reflexes clusters of BNBAS. An association between arsenic and the Motor cluster was demonstrated with an odds ratio of 2.03 in the second timepoint and an odds ratio of 1.36 in the third timepoint, demonstrating an increased odds of sub-optimal performance with elevated arsenic levels. These associations were also demonstrated through linear regression analysis.

Statistically significant associations were found between each of the heavy metals (chromium, arsenic, cadmium, mercury, lead) and the Abnormal Reflexes cluster using Poisson regression. This indicates that there is an increase in the number of observed abnormal reflexes with increasing metabolite levels.

Analyzing the effects of heavy metals exposures during specific timepoints of gestation further supports our findings. By collecting samples at enrollment, a second timepoint (28 weeks gestation), a third timepoint (36 weeks gestation), and cord blood, we are able to obtain samples at important points during and shortly after the pregnancy. During the second trimester, neuronal pathways, control orientation, and refined motor skills are mostly developed. Exposure to neurotoxicants, such as heavy metals, during the formation of these pathways can adversely affect the infant's overall neurobehavioral function after birth. Statistical significance was demonstrated in the correlation between maternal blood samples and cord blood samples in arsenic, mercury, and lead, demonstrating that the developing fetus is exposed to these heavy metals throughout the pregnancy.

Limitations

There were some limitations involved in this study that could impact the results. The sample size was modest at 56 female participants. Thirteen of these women were enrolled in the first trimester of pregnancy, limiting the ability to accurately assess associations between BNBAS outcomes and first trimester heavy metal exposure. Ideally, recruitment would have occurred during the preconception period to have a better assessment of exposure levels during the entire duration of pregnancy and their effects on BNBAS outcomes. There was some variation of the amount of samples collected for each timepoint, but this could have been due to extenuating circumstances. Exposure misclassification may have occurred as a result of variation in exposure measures and a lack of first trimester samples for the majority of women. However, it is likely to be non-differential with respect to the outcome, producing a bias towards the null. Overall, exposure data were very skewed due to a wide variation in exposure between mothers participating in the SAWASDEE cohort.

The measurement of blood heavy metal levels at enrollment and at two separate timepoints represents maternal exposure to pesticide compounds and their associated heavy metals and may not comprehensively evaluate exposure. The use of maternal levels of heavy metals is being used as a proxy for exposure to the fetus in utero. Additionally, it is unknown from the samples where to attribute exposure. Exposure to heavy metals is not exclusively sourced from maternal occupational exposure in the agricultural fields. Study participants may be exposed to heavy metals through other tasks, including in household dust, residential pesticide use, and paint chips. In this cohort, exposures values were

obtained from biological matrices (blood, urine). The use of personal monitoring equipment could have been used to see the most common pathway of exposure and evaluate which pathway is most influential in affecting neurodevelopmental outcomes for the developing fetus.

The ability and accuracy of BNBAS administered at birth to predict future neurological development is largely unknown. Since there was not a follow up assessing neurodevelopmental outcomes in this cohort, it is unknown how these neurobehavioral predictions have manifested in the offspring. It is possible that outcome misclassification may have occurred due to the utilization of a single outcome assessment since BNBAS was administered one time to each infant shortly after birth. Any outcome misclassification that occurred is most likely to be non-differential with respect to exposure, with a bias towards the null.

In the SAWASDEE birth cohort, a variety of important confounders were considered in the present analysis, such as household income, maternal age, maternal education, and maternal pre-pregnancy BMI. However, there were some critical confounders that were unable to be assessed, including parental IQ and exposure to other neurotoxicants, such as alcohol, polychlorinated biphenyls (PCBs), and tobacco smoke. The study population was largely homogenous for household income and maternal education, reducing the potential for uncontrolled confounding of these covariates. As a result, our control of these confounders may not be adequate, resulting in some residual confounding.

Even though limitations are present, this study is pivotal in being one of the first studies to demonstrate how time-specific heavy metal exposures across

the pregnancy impact neurodevelopmental outcomes 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 that these infants are more highly exposed to in utero heavy metals from pesticide residue and perform less optimally on an assessment of neurologic integrity at birth. Therefore, this present study is suggestive of an adverse association between heavy metal exposures as measured by maternal blood and cord blood samples and neurobehavioral functioning at birth. This study demonstrates a great need for additional studies that utilize temporally resolved prenatal exposure data to investigate this association and its applications to populations worldwide.

Recommendations for Future Research

Further researched is needed in order to fully understand how timing of exposure to heavy metals, such as chromium, arsenic, cadmium, mercury, and lead, influence specific aspects of neurodevelopment at birth and throughout the course of childhood. Previous studies have been unable to fully investigate the potential consequences of trimester specific exposures due to the limited number of biological samples collected during pregnancy. Additionally, the present study was unable to unable to thoroughly investigate the impact of first trimester exposure due to a limited number of participants enrolling during their first trimester of pregnancy. Future studies should be designed to incorporate an

accurate quantity of biomarker measures to fully assess in utero exposure throughout the entire course of pregnancy.

In order to understand true exposures, further research would be needed to evaluate occupational versus non-occupational exposure. Future studies should be designed to incorporate personal monitoring equipment for participants to wear to be able to track the source and location of exposures. Additionally, to get a better understanding of non-occupational exposure, samples around the home, such as dust and paint chips, should be collected to enhance exposure source data.

To determine the accuracy of measures of neurodevelopmental outcomes as assessed by BNBAS, further research is needed to follow up on neurodevelopmental patterns throughout childhood. This is important in determining how accurate the predictive measures of BNBAS are in fully assessing neurodevelopmental outcomes throughout the lifespan. Since SAWASDEE is a longitudinal birth cohort, it would be ideal to follow the children as they grow older to assess the accuracy of the BNBAS scores in predicting their neurodevelopmental outcomes.

Policy Recommendations

Prenatal exposure to neurotoxicants, including pesticides and heavy metals, is a public health issue domestically and internationally. In developing countries, such as Thailand, this is an especially concerning issue because regulations regarding the use of personal protective equipment while applying pesticides are absent or unenforced. Developed countries, such as the United

States, are able to research this public health issue. Fortunately, there are a number of studies that have demonstrated replicable scientific evidence showing a relationship to low-level exposure to heavy metals and a range of adverse health outcomes, including neurodevelopment (Yu, Yan et al. 2011, Zheng, Zhong et al. 2014).

The Toxic Substances Control Act (TSCA) of 1976 provides the currently regulatory framework for chemicals in the United States, utilizing a risk assessment model to evaluate and determine safety. While monumental for its time, serving as the first regulatory framework worldwide, TSCA is outdated for the modern era of chemical regulation. The risk-based model that TSCA utilizes does not account for the low dose exposures that can have adverse health effects. Instead, TSCA solely focuses on the extreme doses and overt adverse health effects. Since the framework of TSCA uses less sensitive endpoints, it does not adequately protect infants and children from adverse neurodevelopmental effects, who are more highly exposed to harmful substances per unit volume than adults (Landrigan 2011).

The SAWASDEE birth cohort will hopefully serve as a foundation to inform future policy because it is the first study to have time-resolved pregnancy exposure data. This pilot study, along with our planned future studies in Thailand, will be influential for regulatory reform for heavy metal exposures from OP pesticides to be more protective for maternal and children's environmental health.

VI. REFERENCES

ATSDR Lead. E. D. Management. Atlanta, GA.

ATSDR (2012). Toxicological Profile for Cadmium. Atlanta, GA, U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry (ATSDR).

ATSDR (2012). Toxicological Profile for Chromium. U. S. D. o. H. a. H. Services. Atlanta, GA.

Braun JM, H. E., Schwartz J, Sanchez B, Schnaas L, Mercado-Garcia A,, et al. (2012). "Assessing windows of susceptibility to lead-induced cognitive deficits in Mexican children." Neurotoxicology **33**(5): 1040-1047.

Brazelton, T. B. (1978). "Organization and Stability of Newborn Behavior- A Commentary on the Brazelton Neonatal Behavior Assessment Scale " Society for Research in Child Development **43**(5/6): 1-13.

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

Brenda Eskenazi, K. H., Amy Marks, Kim G. Harley, Asa Bradman, Dana Boyd Barr, Nina Holland (2010). "PON1 and Neurodevelopment in Children from the CHAMACOS Study Exposed to Organophosphate Pesticides in Utero." Environ Health Perspect **118**: 1775-1781.

Carol J. Burns, L. J. M., Pamela J. Mink, Anne M. Jurek, and Abby A. Li (2013). "Pesticide Exposure and Neurodevelopmental Outcomes: Review of the Epidemiologic and Animal Studies." Journal of Toxicology and Environmental Health **16**(3-4): 127-283.

CDC (2009). Organophosphorus Insecticides: Dialkyl Phosphate Metabolites. N. C. f. E. Health. Atlanta, GA, Department of Health and Human Services.

CDC (2013). "Biomonitoring Summary: Organophosphorus Insecticides: Dialkyl Phosphate Metabolites." Retrieved 3/10/15, 2015, from http://www.cdc.gov/biomonitoring/OP-DPM_BiomonitoringSummary.html.

Ciesielski, T., Weuve, Jennifer , Bellinger, David C., Schwartz, Joel, Lanphear, Bruce, and Robert O. Wright (2012). "Cadmium Exposure and Neurodevelopmental Outcomes in U.S. Children." Environ Health Perspect **120**(5): 758-763.

D. Caserta, A. G., G. Lo Monte, G. Bordi, M. Moscarini (2013). "Heavy metals and placental fetal-matenral barrier: a mini-review on the major concerns." European Review for Medical and Pharmacological Sciences **17**: 2198-2206.

Deroma L, P. M., Tognin V, Channoufi L, Tratnik J, Horvat M, Valent F, and B. F. (2013). "Neuropsychological assessment at school-age and prenatal low-level exposure to mercury through fish consumption in an Italian birth cohort living near a contaminated site." Int J Hyg Environ Health **216**(4): 486-493.

EPA, U. S. (2007). Chemical Summary Form, Inorganic Arsenic. U. S. EPA. Washington, D.C.

EPA, U. S. (2014). "Lead." Retrieved 9/22/14, 2014.

Garbo G, F. M., Ivaldi PA, Caroni G, Ferrari G, Giachino GM. (1998). "[Lead poisoning in the newborn and infants: an epidemiological study in an area of Northern Italy]." Pediatr Med Chir. **20**(5): 309-313.

Grandjean P, W. P., Debes F, Choi AL, Budtz-Jørgensen E (2014). "Neurotoxicity from prenatal and postnatal exposure to methylmercury." Neurotoxicol and Teratology **43**: 39-44.

Jessica G. Young, B. E., Eleanor A. Gladstone, Asa Bradman, Lesley Pedersen, Caroline Johnson, Dana B. Barr, Clement E. Furlong, Nina T. Holland (2005). "Association Between In Utero Organophosphate Pesticide Exposure and Abnormal Reflexes in Neonates." Neurotoxicology **26**(2): 199-209.

Landrigan, P. J. a. G., Lynn R. (2011). "Children's Vulnerability to Toic Chemicals: A Challenge and Opportunity to Strengthen Health and Environmental Policy." Health Affairs **30**(5): 842-850.

Lorenz, A. N., et al. (2012). "Pilot study of pesticide knowledge, attitudes, and practices among pregnant women in northern Thailand." Int J Environ Res Public Health **9**(9): 3365-3383.

María Teresa Muñoz-Quezada, B. A. L., Dana B. Barr, Kyle Steenland, Karen Levy, P. Barry Ryan, Veronica Iglesias, Sergio Alvarado, Carlos Concha, Evelyn and C. V. Rojas (2013). "Neurodevelopmental effects in children associated with exposure to organophosphate pesticides: A Systematic review." Neurotoxicology **39**: 158-168.

Matthews, E. K. (2014). Prenatal Organophosphate Pesticide Exposure and Neurodevelopmental Outcomes in a Thai Agricultural Birth Cohort. Environmental Health. Atlanta, GA, Emory University. **MPH**.

McDermott, S., et al. (2014). "Are different soil metals near the homes of pregnant women associated with mild and severe intellectual disability in children?" Dev Med Child Neurol **56**(9): 888-897.

Michael Lewis, J. W., Douglas Ramsay, Michael McCormack (1992). "Prenatal Exposure to Heavy Metals: Effect on Childhood Cognitive Skills and Health Status." Pediatrics **89**(6): 1010-1015.

Murata K, B.-J. E., Grandjean P. (2002). "Benchmark dose calculations for methylmercury-associated delays on evoked potential latencies in two cohorts of children." Risk Analysis **22**(3): 465-474.

Myers GJ, M. D., Cox C, Davidson PW, Shamlaye CF, Tanner MA, Choi A, and C. O. Cernichiari E, Clarkson TW. (1995). "A pilot neurodevelopmental study of Seychellois children following in utero exposure to methylmercury from a maternal fish diet." Neurotoxicology **16**(4): 629-638.

Nyanza, E., Joseph, M, Premji, SS, Thomas, DS, Mannion, C (2014). "Geophagy practices and the content of chemical elements in the soil eaten by pregnant women in artisanal and small scale gold mining communities in Tanzania." BMC Pregnancy and Childbirth **144**: 1-10.

Tchounwou, P. B., Yedjou, Clement G., Patlolla, Anita K., Sutton, Dwayne J. (2012). Heavy Metals Toxicity and the Environment. D. o. H. a. H. Services.

UN (2014). "Men and Women in Agriculture: Closing the Gap." Retrieved 12/4/14, 2014.

USDA (2000). Heavy Metal Soil Contamination. Soil Quality--Urban Technical Note, United States Department of Agriculture (USDA).

Yorifuji T, D. F., Weihe P, Grandjean P. (2011). "Prenatal exposure to lead and cognitive deficit in 7- and 14-year-old children in the presence of concomitant exposure to similar molar concentration of methylmercury." Neurotoxicol and Teratology **33**(2): 205-211.

Yu, X. D., et al. (2011). "Prenatal exposure to multiple toxic heavy metals and neonatal neurobehavioral development in Shanghai, China." Neurotoxicol Teratol **33**(4): 437-443.

Zheng, G., et al. (2014). "Levels of heavy metals and trace elements in umbilical cord blood and the risk of adverse pregnancy outcomes: a population-based study." Biol Trace Elem Res **160**(3): 437-444.

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%
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		
1,500 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%
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
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)

Metal of Exposure	*n*	*Mean (ng/mL)*	*Median (ng/mL)*	*SD (ng/mL)*	*Range (ng/mL)*	*IQR (ng/mL)*
Chromium						
At enrollment	41	0.6	0.3	0.7	<LOQ-3.6	0.4
At Second Timepoint	42	0.9	0.4	1.8	<LOQ-8.7	0.3
At Third Timepoint	48	1.7	0.8	4.0	<LOQ – 25.2	1.3
Cord Blood	33	0.7	0.4	1.2	0.03-6.8	0.3
Arsenic						
At enrollment	55	2.1	1.6	2.4	1.0 – 18.5	0.6
At Second Timepoint	50	1.8	1.5	1.0	0.8 – 6.2	0.8
At Third Timepoint	51	2.4	1.6	3.5	0.7 – 24.3	1.1
Cord Blood	45	1.7	1.3	1.5	0.4 - 10.2	0.9
Cadmium						
At enrollment	55	0.8	0.7	0.4	0.2 – 2.3	0.3
At Second Timepoint	50	0.7	0.6	0.4	0.2 – 1.8	0.4
At Third Timepoint	51	0.8	0.6	0.4	0.1 - 1.8	0.4
Cord Blood	45	0.1	0.1	0.1	<LOQ – 0.6	<LOQ
Mercury						
At enrollment	55	1.4	1.2	0.8	0.2 – 4.8	1.0
At Second Timepoint	50	1.2	1.2	0.6	0.1 - 3.2	0.9
At Third Timepoint	48	1.2	1.2	0.9	0.1 - 4.5	0.9
Cord Blood	45	2.1	1.8	1.5	<LOQ – 8.0	1.7
Lead						
At enrollment	55	29.6	26.8	12.9	8.7 – 66.8	12.7
At Second Timepoint	50	26.2	22.8	14.1	9.9 – 88.6	12.8
At Third Timepoint	51	27.1	23.1	13.5	8.7 - 68.7	15.0
Cord Blood	45	20.5	17.5	12.8	2.9 – 70.6	9.9

Table 4. Correlation of Maternal Chromium Levels and Cord Blood

	Enrollment	Second Timepoint	Third Timepoint	Cord Blood
Enrollment				
Correlation	1.00	-0.09	-0.09	-0.05
p-value	n/a	0.60	0.60	0.80
Second Timepoint				
Correlation	-0.09	1.00	-0.10	0.04
p-value	0.60	n/a	0.52	0.84
Third Timepoint				
Correlation	-0.09	-0.10	1.00	0.10
p-value	0.60	0.52	n/a	0.57
Cord Blood				
Correlation	-0.04	0.04	0.10	1.00
p-value	0.80	0.84	0.57	n/a

Table 5. Correlation of Maternal Arsenic Levels and Cord Blood

	Enrollment	Second Timepoint	Third Timepoint	Cord Blood
Enrollment				
Correlation	1.00	0.45	0.03	0.06
p-value	n/a	0.001	0.84	0.71
Second Timepoint				
Correlation	0.45	1.00	0.10	0.15
p-value	0.001	n/a	0.48	0.32
Third Timepoint				
Correlation	0.03	0.10	1.00	0.64
p-value	0.84	0.48	n/a	<0.0001
Cord Blood				
Correlation	0.06	0.15	0.64	1.00
p-value	0.71	0.32	<0.0001	n/a

Table 6. Correlation of Maternal Cadmium Levels and Cord Blood

	Enrollment	Second Timepoint	Third Timepoint	Cord Blood
Enrollment				
Correlation	1.00	0.90	0.88	0.08
p-value	n/a	<0.0001	<0.0001	0.59
Second Timepoint				
Correlation	0.90	1.00	0.92	0.001
p-value	<0.0001	n/a	<0.0001	1.00
Third Timepoint				
Correlation	0.88	0.92	1.00	-0.14
p-value	<0.0001	<0.0001	n/a	0.36
Cord Blood				
Correlation	0.08	0.001	-0.14	1.00
p-value	0.59	1.00	0.36	n/a

Table 7. Correlation of Maternal Mercury Levels and Cord Blood

	Enrollment	Second Timepoint	Third Timepoint	Cord Blood
Enrollment				
Correlation	1.00	0.52	0.51	0.25
p-value	n/a	<0.0001	0.0002	0.10
Second Timepoint				
Correlation	0.52	1.00	0.45	0.60
p-value	<0.0001	n/a	0.002	<0.0001
Third Timepoint				
Correlation	0.51	0.45	1.00	0.38
p-value	0.0002	0.002	n/a	0.01
Cord Blood				
Correlation	0.25	0.60	0.38	1.00
p-value	0.10	<0.0001	0.01	n/a

Table 8. Correlation of Maternal Lead Levels and Cord Blood

	Enrollment	Second Timepoint	Third Timepoint	Cord Blood
Enrollment				
Correlation	1.00	0.78	0.77	0.70
p-value	n/a	<0.0001	<0.0001	<0.0001
Second Timepoint				
Correlation	0.78	1.00	0.89	0.90
p-value	<0.0001	n/a	<0.0001	<0.0001
Third Timepoint				
Correlation	0.77	0.89	1.00	0.88
p-value	<0.0001	<0.0001	n/a	<0.0001
Cord Blood				
Correlation	0.70	0.90	0.88	1.00
p-value	<0.0001	<0.0001	<0.0001	n/a

Table 9. Correlation Coefficients for Enrollment Samples

	Chromium	Arsenic	Cadmium	Mercury	Lead
Chromium					
Correlation	1.00	-0.14	0.01	-0.10	-0.16
p-value	n/a	0.38	0.93	0.55	0.33
Arsenic					
Correlation	-0.14	1.00	0.03	-0.08	-0.03
p-value	0.38	n/a	0.82	0.57	0.81
Cadmium					
Correlation	0.01	0.03	1.00	0.15	-0.01
p-value	0.93	0.81	n/a	0.28	0.96
Mercury					
Correlation	-0.10	-0.08	0.15	1.00	0.09
p-value	0.54	0.57	0.28	n/a	0.51
Lead					
Correlation	-0.16	-0.03	-0.01	0.09	1.00
p-value	0.33	0.81	0.96	0.51	n/a

Table 10. Correlation Coefficients for Second Timepoint Samples

	Chromium	Arsenic	Cadmium	Mercury	Lead
Chromium					
Correlation	1.00	-0.23	-0.01	-0.06	-0.02
p-value	n/a	0.15	0.96	0.72	0.89
Arsenic					
Correlation	-0.23	1.00	0.22	0.15	0.14
p-value	0.15	n/a	0.12	0.30	0.33
Cadmium					
Correlation	-0.01	0.22	1.00	0.12	0.06
p-value	0.96	0.12	n/a	0.40	0.70
Mercury					
Correlation	-0.06	0.15	0.12	1.00	0.08
p-value	0.72	0.30	0.40	n/a	0.58
Lead					
Correlation	-0.02	0.14	0.06	0.08	1.00
p-value	0.89	0.33	0.70	0.58	n/a

Table 11. Correlation Coefficients for Third Timepoint Samples

	Chromium	Arsenic	Cadmium	Mercury	Lead
Chromium					
Correlation	1.00	-0.08	-0.02	-0.08	0.18
p-value	n/a	0.59	0.88	0.61	0.23
Arsenic					
Correlation	-0.08	1.00	-0.01	-0.11	-0.10
p-value	0.59	n/a	0.95	0.43	0.46
Cadmium					
Correlation	-0.02	-0.01	1.00	-0.09	0.13
p-value	0.88	0.95	n/a	0.54	0.36
Mercury					
Correlation	-0.08	-0.12	-0.09	1.00	0.02
p-value	0.61	0.43	0.54	n/a	0.89
Lead					
Correlation	0.18	-0.10	0.13	0.02	1.00
p-value	0.23	0.46	0.36	0.89	n/a

Table 12. Correlation Coefficients for Cord Blood Samples

	Chromium	Arsenic	Cadmium	Mercury	Lead
Chromium					
Correlation	1.00	-0.12	-0.17	0.11	0.01
p-value	n/a	0.50	0.35	0.53	0.97
Arsenic					
Correlation	-0.12	1.00	0.15	0.08	-0.03
p-value	0.50	n/a	0.33	0.61	0.84
Cadmium					
Correlation	-0.17	0.15	1.00	-0.25	-0.09
p-value	0.35	0.33	n/a	0.10	0.55
Mercury					
Correlation	0.11	0.08	-0.25	1.00	0.16
p-value	0.53	0.61	0.10	n/a	0.31
Lead					
Correlation	0.01	-0.03	-0.09	0.16	1.00
p-value	0.97	0.84	0.55	0.31	n/a

Table 13. 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	54	38.0	39.0	7.3	14.0 – 51.0	34.0 – 44.0	63
Motor	54	38.4	39.0	6.9	14.0 – 51.0	34.0 – 44.0	45
Range of State	54	16.0	16.0	1.8	13.0 – 22.0	15.0 – 17.0	36
Regulation of State	55	16.4	16.0	2.7	13.0 – 26.0	15.0 – 18.0	36
Autonomic Stability	54	19.5	19.5	1.6	15.0 – 21.0	19.0 – 21.0	27
Abnormal Reflexes	54	1.11	1.00	1.24	0.00 – 6.00	0.00 – 1.00	n/a

Table 14. Association between enrollment average heavy metal analyte levels and summed scores for six BNBAS clusters

BNBAS Cluster	Enrollment Chromium			Enrollment Arsenic			Enrollment Cadmium			Enrollment Mercury			Enrollment Lead		
	N	*β*	*95% CI*	*N*	*β*	*95% CI*	*N*	*β*	*95% CI*	*N*	*β*	*95% CI*	*N*	*β*	*95% CI*
Habituation[a]	40	-0.38	-1.43, 0.67	40	-0.30	-1.56, 0.97	40	-0.007	-1.61, 1.60	40	-0.09	-0.97, 0.80	40	0.04	-0.02, 0.1
Orientation[a]	40	-0.26	-3.51, 2.99	40	1.22	-2.71, 5.14	40	-4.22	-9.21, 0.78	40	-0.88	-3.61, 1.86	40	-0.16	-3.61, 1.86
Motor[b]	39	-0.05	-3.50, 3.40	39	1.14	-3.12, 5.40	39	-4.27	-9.51, 0.98	39	-0.66	-3.69, 2.37	39	-0.17	-0.37, 0.04
Range of State[b]	38	-0.46	-1.32, 0.40	38	0.65	-0.41, 1.72	38	0.39	-0.92, 1.70	38	0.12	-0.64, 0.88	38	0.02	-0.04, 0.13
Regulation of State[b]	38	0.01	-1.29, 1.30	38	0.92	-0.68, 2.52	38	0.90	-1.07, 2.87	38	-0.01	-1.15, 1.12	38	0.02	-0.05, 0.09
Autonomic Stability[c]	38	-0.14	-0.89, 0.60	38	0.13	-0.79, 1.05	38	-0.89		-2.04, 0.26	-0.56	-1.20, 0.08	38	0.004	-0.04, 0.05

[a] adjusted for maternal age, maternal education
[b] adjusted for maternal age, maternal education, household income
[c] adjusted for maternal age, household income

Table 15. Association between second timepoint average heavy metal analyte levels and summed scores for six BNBAS clusters

BNBAS Cluster	Second Timepoint Chromium			Second Timepoint Arsenic			Second Timepoint Cadmium			Second Timepoint Mercury			Second Timepoint Lead		
	N	*β*	*95% CI*	*N*	*β*	*95% CI*	*N*	*β*	*95% CI*	*N*	*β*	*95% CI*	*N*	*β*	*95% CI*
Habituation[a]	42	-0.20	-0.69, 0.29	42	-1.75	-2.79, -0.70	42	0.19	-2.44, 2.83	42	0.57	-0.94, 2.08	42	0.09	0.01, 0.17
Orientation[b]	42	-0.52	-1.93, 0.89	42	-1.14	-3.91, 1.64	42	-1.35	-8.82, 6.13	42	-2.42	-6.77, 1.94	42	-0.12	-0.35, 0.11
Motor[c]	41	-0.72	-2.04, 0.61	41	-1.71	-4.57, 1.15	41	-2.48	-9.57, 4.60	41	-1.95	-6.13, 2.23	41	-0.10	-0.32, 0.12
Range of State[d]	39	0.03	-0.26, 0.32	39	1.06	0.43, 1.69	39	0.86	-0.87, 2.60	39	-0.50	-1.41, 0.41	39	0.01	-0.04, 0.06
Regulation of State[c]	42	-0.12	-0.59, 0.34	42	0.46	-0.56, 1.48	42	1.06	-1.48, 3.6-	42	-0.18	-1.67, 1.13	42	0.01	-0.08, 0.10
Autonomic Stability[d]	41	-0.15	-0.42, 0.13	41	-0.69	-1.29, -0.10	41	-1.24	-2.89, 0.41	41	0.28	-0.59, 1.15	41	-0.01	-0.06, 0.17

[a] adjusted for maternal education
[b] adjusted for maternal age
[c] adjusted for maternal age, household income
[d] adjusted for maternal age, maternal education, household income

Table 16 Association between third timepoint average heavy metal analyte levels and summed scores for six BNBAS clusters

BNBAS Cluster	Third Timepoint Chromium			Third Timepoint Arsenic			Third Timepoint Cadmium			Third Timepoint Mercury			Third Timepoint Lead		
	N	*β*	*95% CI*	*N*	*β*	*95% CI*	*N*	*β*	*95% CI*	*N*	*β*	*95% CI*	*N*	*β*	*95% CI*
Habituation[a]	42	0.04	-0.17, 0.28	42	0.10	-0.14, 0.35	42	-0.13	-2.76, 2.50	42	-0.14	-1.25, 0.98	42	0.04	-0.03, 0.11
Orientation[a]	42	-0.22	-0.75, 0.32	42	-1.06	-1.65, -0.48	42	-0.19	-6.65, 6.28	42	-1.16	-3.82, 1.50	42	-0.16	-0.36, 0.04
Motor[a]	41	-0.24	-0.69, 0.20	41	-1.13	-1.61, -0.64	41	-1.65	-7.09, 3.79	41	-0.14	-3.35, 1.08	41	-0.14	-0.30, 0.03
Range of State[a]	41	-0.04	-0.18, 0.11	41	0.05	-0.11, 0.21	41	1.11	-0.66, 2.87	41	0.03	-0.69, 0.75	41	-0.01	-0.04, 0.07
Regulation of State[b]	42	0.09	-0.11, 0.28	42	-0.05	-0.27, 016	42	1.87	-0.35, 4.09	42	0.11	-0.86, 1.09	42	-0.01	-0.08, 0.06
Autonomic Stability[b]	41	-0.05	-0.16, 0.06	41	0.02	-0.11, 0.14	41	-1.94	-3.26, -0.62	41	-0.01	-0.59, 0.56	41	0.01	-0.03, 0.05

[a] adjusted for maternal age, maternal education, household income

[b] adjusted for maternal education, household income

Table 17. Association between cord blood average heavy metal analyte levels and summed scores for six BNBAS clusters

BNBAS Cluster	Cord Blood Chromium			Cord Blood Arsenic			Cord Blood Cadmium			Cord Blood Mercury			Cord Blood Lead		
	N	*β*	*95% CI*	*N*	*β*	*95% CI*	*N*	*β*	*95% CI*	*N*	*β*	*95% CI*	*N*	*β*	*95% CI*
Habituation[a]	33	-0.13	-1.16, 0.91	33	-0.08	-0.84, 0.67	33	3.27	-6.34, 12.90	33	0.36	-0.78, 1.49	33	0.06	-0.04, 0.15
Orientation[b]	31	0.08	-2.50, 2.65	31	-0.44	-2.33, 1.45	31	-16.42	-40.51, 7.67	31	1.22	-1.86, 4.30	31	-0.12	-0.44, 0.21
Motor[b]	30	-0.29	-2.50, 1.92	30	-0.88	-2.52, 0.77	30	-21.21	-42.05, -0.38	30	0.49	-2.19, 3.16	30	-0.09	-0.36, 0.19
Range of State[c]	32	-0.52	-1.08, 0.04	32	0.01	-0.40, 0.43	32	-0.34	-5.54, 4.87	32	-0.40	-1.02, 0.22	32	0.05	0.0004, 0.10
Regulation of State[d]	31	-0.34	-0.97, 0.30	31	-0.02	-0.49, 0.45	31	-5.18	-11.13, 0.77	31	-0.87	-1.60, -0.13	31	0.03	-0.05, 0.10
Autonomic Stability[d]	30	0.30	-0.19, 0.79	30	0.01	-0.37, 0.37	30	2.82	-1.83, 7.48	30	0.48	-0.09, 1.06	30	-0.03	-0.09. 0.03

[a] adjusted for maternal age, maternal education
[b] adjusted for maternal age, maternal education, household income
[c] adjusted for maternal age
[d] adjusted for maternal age, household income

Table 18 Association between average heavy metal metabolites and abnormal reflexes

	Chromium			**Arsenic**			**Cadmium**			**Mercury**			**Lead**		
	N	*β*	*95% CI*	*N*	*β*	*95% CI*	*N*	*β*	*95% CI*	*N*	*β*	*95% CI*	*N*	*β*	*95% CI*
Enrollment[a]	39	0.99	0.60, 1.62	39	1.06	0.60, 1.85	39	1.05	1.12, 3.77	39	0.78	0.50, 1.21	39	0.99	0.97, 1.02
Second Timepoint[a]	41	0.89	0.69, 1.16	41	1.07	0.74, 1.55	41	1.60	0.54, 4.75	41	0.80	0.42, 1.52	41	0.99	0.96, 1.02
Third Timepoint[b]	41	1.00	0.92, 1.09	41	1.09	1.04, 1.12	41	1.32	0.56, 3.10	41	0.96	0.61, 1.49	41	1.00	0.97, 1.03
Cord Blood[b]	32	0.66	0.30, 1.45	32	1.07	0.90, 1.28	32	4.22	0.40, 44.81	32	0.84	0.55, 1.27	32	1.02	0.96, 1.08

[a] adjusted for maternal education

[b] adjusted for household income

VIII. APPENDICES

A. Methods for Scoring BNBAS

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 Assessme
Scale (4th ed.). London: Mac Keith Press.

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	❑	❑	❑	❑	❑	_____

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.

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 enrollment average heavy metal levels and average scores for seven BNBAS Clusters

BNBAS Cluster	Chromium			Arsenic			Cadmium			Mercury			Lead		
	N	*OR*	*95% CI*	*N*	*OR*	*95% CI*	*N*	*OR*	*95% CI*	*N*	*OR*	*95% CI*	*N*	*OR*	*95% C*
Habituation[a]	40	1.36	0.60, 3.08	40	1.13	0.42, 3.03	40	0.87	0.25, 3.06	40	1.16	0.59, 2.31	40	0.97	0.92, 1.01
Orientation[b]	40	1.20	0.54, 2.68	40	0.59	0.22, 1.58	40	2.32	0.65, 8.30	40	1.45	0.74, 2.85	40	1.05	1.00, 1.10
Motor[c]	38	1.25	0.56, 2.79	38	0.51	0.18, 1.39	38	2.81	0.78, 10.16	38	1.61	0.81, 3.18	38	1.03	0.98, 1.09
Range of State[b]	40	1.94	0.80, 4.62	40	0.56	0.20, 1.54	40	0.62	0.17, 2.25	40	0.68	0.34, 1.36	40	1.01	0.96, 1.06
Regulation of State[d]	40	1.45	0.65, 3.26	40	0.56	0.21, 1.52	40	0.88	0.25, 3.04	40	1.38	0.70, 2.70	40	0.99	0.94, 1.03
Autonomic Stability[a]	40	1.04	0.43, 2.47	40	0.74	0.26, 2.06	40	2.31	0.62, 8.63	40	1.84	0.88, 3.83	40	0.99	0.94, 1.04
Abnormal Reflexes[b]	40	1.01	0.41, 2.46	40	0.65	0.22, 1.91	40	0.24	0.06, 0.96	40	1.66	0.76, 3.64	40	1.01	0.96, 1.06

[a] adjusted for maternal age, maternal education
[b] adjusted for gestational age, maternal education
[c] adjusted for gestational age, household income
[d] adjusted for gestational age

Table 2. Association between second timepoint average heavy metal levels and average scores for seven BNBAS Clusters

BNBAS Cluster	Chromium			Arsenic			Cadmium			Mercury			Lead		
	N	*OR*	*95% CI*	*N*	*OR*	*95% CI*	*N*	*OR*	*95% CI*	*N*	*OR*	*95% CI*	*N*	*OR*	*95% C*
Habituation[a]	42	1.10	0.80, 1.51	42	1.76	0.88, 3.49	42	0.68	0.11, 4.21	42	0.88	0.22, 2.33	42	0.96	0.90, 1.01
Orientation[b]	42	1.26	0.92, 1.73	42	1.76	0.94, 3.29	42	1.76	0.34, 9.16	42	1.93	0.73, 5.10	42	1.05	1.00, 1.11
Motor[b]	41	1.34	0.97, 1.85	41	2.03	1.06, 3.87	41	2.11	0.40, 11.16	41	2.00	0.75, 5.32	41	1.06	1.00, 1.11
Range of State[b]	41	0.94	0.68, 1.29	41	0.31	0.15, 0.65	41	0.41	0.07, 2.30	41	2.04	0.75, 5.57	41	1.01	0.96, 1.06
Regulation of State[b]	42	1.09	0.79, 1.50	42	0.49	0.26, 0.95	42	0.72	0.14, 3.80	42	1.71	0.64, 4.57	42	0.99	0.87, 1.13
Autonomic Stability	41	1.37	0.97, 1.94	41	3.25	1.51, 6.99	41	7.19	1.06, 48.74	41	0.47	0.16, 1.39	41	1.01	0.95, 1.06
Abnormal Reflexes[c]	39	1.27	0.84, 1.92	39	0.81	0.37, 1.76	39	0.52	0.08, 3.48	39	0.52	0.16, 1.65	39	1.00	0.93, 1.06

[a] adjusted for maternal age, maternal education
[b] adjusted for maternal age
c adjusted for gestational age, maternal education, household income

Table 3. Association between third timepoint average heavy metal levels and average scores for seven BNBAS Clusters

BNBAS Cluster	Chromium			Arsenic			Cadmium			Mercury			Lead		
	N	*OR*	*95% CI*	*N*	*OR*	*95% CI*	*N*	*OR*	*95% CI*	*N*	*OR*	*95% CI*	*N*	*OR*	*95% CI*
Habituation[a]	44	0.96	0.83, 1.10	44	0.78	0.65, 0.94	44	0.58	0.12, 2.71	44	1.00	0.51, 1.94	44	0.97	0.93, 1.02
Orientation[b]	42	1.15	1.00, 1.32	42	1.31	1.08, 1.58	42	1.53	0.36, 6.49	42	1.38	0.71, 2.70	42	1.05	1.00, 1.10
Motor[b]	41	1.14	0.99, 1.31	41	1.36	1.11, 1.66	41	1.84	0.43, 7.91	41	1.41	0.73, 2.75	41	1.05	1.01, 1.10
Range of State[c]	43	1.06	0.92, 1.20	43	0.97	0.84, 1.13	43	0.65	0.14, 2.94	43	1.08	0.56, 2.07	43	0.98	0.94, 1.02
Regulation of State[d]	44	0.93	0.81, 1.07	44	1.07	0.92, 1.25	44	0.47	0.12, 1.94	44	1.03	0.54, 2.00	44	0.99	0.96, 1.03
Autonomic Stability[e]	43	1.11	0.96, 1.28	43	0.95	0.80, 1.13	43	11.45	2.14, 61.35	43	1.08	0.52, 2.24	43	0.98	0.94, 1.02
Abnormal Reflexes[c]	43	1.00	0.87, 1.15	43	0.72	0.58, 0.92	43	0.28	0.05, 1.50	43	0.99	0.48, 2.05	43	1.06	0.92, 1.22

[a] adjusted for maternal age, maternal education, household income
[b] adjusted for gestational age, household income
[c] adjusted for maternal age
[d] adjusted for gestational age
[e] adjusted for gestational age, maternal education

Table 4. Association between cord blood average heavy metal levels and average scores for seven BNBAS Clusters

BNBAS Cluster	Chromium			Arsenic			Cadmium			Mercury			Lead		
	N	*OR*	*95% CI*	*N*	*OR*	*95% CI*	*N*	*OR*	*95% CI*	*N*	*OR*	*95% CI*	*N*	*OR*	*95% C*
Habituation[a]	33	1.22	0.73, 2.05	33	1.16	0.79, 1.70	33	0.04	<0.001, 5.52	33	0.64	-0.35, 1.15	33	0.96	0.91, 1.01
Orientation[a]	33	1.19	0.71, 1.97	33	1.18	0.81, 1.73	33	207.50	0.78, >999.99	33	1.07	0.60, 1.89	33	1.01	0.96, 1.05
Motor[a]	32	1.21	0.72, 2.01	32	1.23	0.83, 1.80	32	261.85	1.37, >999.99	32	1.09	0.61, 1.92	32	1.01	0.96, 1.06
Range of State[a]	32	2.34	1.10, 4.98	32	1.02	0.69, 1.51	32	1.13	0.01, 141.80	32	1.60	0.88, 2.94	32	0.95	0.90, 1.00
Regulation of State[a]	33	1.37	0.80, 2.34	33	1.04	0.71, 1.12	33	176.57	1.03, >999.99	33	2.23	1.22, 4.42	33	0.98	0.93, 1.02
Autonomic Stability[a]	32	0.75	0.42, 1.34	32	1.04	0.71, 1.54	32	0.04	<0.001, 6.69	32	0.61	0.22, 1.13	32	1.00	0.95, 1.05
Abnormal Reflexes[a]	32	1.50	0.69, 3.20	32	0.86	0.57, 1.29	32	0.11	<0.001, 19.28	32	0.96	0.51, 1.80	32	1.00	0.95, 1.05

[a] adjusted for gestational age, maternal age

Table 5. Comparative Studies Evaluating Prenatal Heavy Metal Exposure and Neurological Impacts in Populations of High Exposure

Cohort	Years	Citation	Metals	Effect
Faroese birth cohort	1986-1987	Grandjean P, Weihe P, Debes F, Choi AL, Budtz-Jørgensen E. Neurotoxicity from prenatal and postnatal exposure to methylmercury. Neurotoxicol Teratol. 2014 May-Jun;43:39-44.	Methylmercury	Neurodevelopmental deficiencies, especially a decrease in visuospatial memory
Faroese birth cohort	1986-1987	Yorifuji T, Debes F, Weihe P, Grandjean P. Prenatal exposure to lead and cognitive deficit in 7- and 14-year-old children in the presence of concomitant exposure to similar molar concentration of methylmercury. Neurotoxicol Teratol. 2011 Mar-Apr;33(2):205-11.	Lead, Methylmercury	Greater cognitive deficiencies with elevated levels of lead with co-exposure to methylmercury
Farose and Madeiran birth cohorts	1986-1987	Murata K, Budtz-Jørgensen E, Grandjean P. Benchmark dose calculations for methylmercury-associated delays on evoked potential latencies in two cohorts of children. Risk Anal. 2002 Jun;22(3):465-74.	Methylmercury	Methylmercury associated with delays on evoked potential latencies
International birth cohorts	1989-2003	Lanphear BP, Hornung R, Khoury J, Yolton K, Baghurst P, Bellinger DC, Canfield RL, Dietrich KN, Bornschein R, Greene T, Rothenberg SJ, Needleman HL, Schnaas L, Wasserman G, Graziano J, Roberts R. Low-level	Lead	Intellectual deficits

		environmental lead exposure and children's intellectual function: an international pooled analysis. Environ Health Perspect. 2005 Jul;113(7):894-9.		
Italian birth cohort (Northeastern Italy)	2001	Deroma L, Parpinel M, Tognin V, Channoufi L, Tratnik J, Horvat M, Valent F, Barbone F. Neuropsychological assessment at school-age and prenatal low-level exposure to mercury through fish consumption in an Italian birth cohort living near a contaminated site. Int J Hyg Environ Health. 2013 Jul;216(4):486-93.	Mercury	Higher mercury levels lead to lower IQ scores
Italian birth cohort (Northern Italy)	Prior to 1998†	Garbo G, Frigerio M, Ivaldi PA, Caroni G, Ferrari G, Giachino GM. [Lead poisoning in the newborn and infants: an epidemiological study in an area of Northern Italy]. Pediatr Med Chir. 1998 Sep-Oct;20(5):309-13.	Lead	Lead can cross the placenta and is present in both rural and urban populations
Mexico City birth cohorts	1994-2007	Braun JM, Hoffman E, Schwartz J, Sanchez B, Schnaas L, Mercado-Garcia A, Solano-Gonzalez M, Bellinger DC, Lanphear BP, Hu H, Tellez-Rojo MM, Wright RO, Hernandez-Avila M. Assessing windows of susceptibility to lead-induced cognitive deficits in Mexican children. Neurotoxicology. 2012	Lead	Decreased cognitive abilities

	Oct;33(5):1040-7.		
Seychelles birth cohort	Myers GJ, Marsh DO, Cox C, Davidson PW, Shamlaye CF, Tanner MA, Choi A, Cernichiari E, Choisy O, Clarkson TW. A pilot neurodevelopmental study of Seychellois children following in utero exposure to methylmercury from a maternal fish diet. Neurotoxicology. 1995 Winter;16(4):629-38.	Methylmercury	Fetal mercury exposure caused abnormal developmental effects

† Only had access to the abstract as the article was in an Italian journal and the years of the study were not given in the abstract.