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Prenatal Organochlorine Exposure and Neurobehavioral Performance in a Thai Agricultural Birth Cohort

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Master of Science in Public Health

Global Epidemiology

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Prenatal Organochlorine Exposure and Neurobehavioral Performance in a Thai Agricultural Birth Cohort

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Abstract

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By Ashley A. Horne

Background: DDE is a metabolite of DDT, an organochlorine pesticide associated with acute and long term health effects which is restricted in some countries and still in use in others. DDE can persist in the environment decades after use has stopped, and can travel via wind and water. As a result, use in one geographical area can affect a population and environment in other places with restricted or prohibited use. Exposure of pregnant women to organochlorine pesticides is of particular concern, since the developing fetus is known to be more susceptible to harmful effects of toxic chemicals than the general population. Our study aims to fill the current gap in literature which examines prenatal exposure to DDT and its metabolites using repeated measures of exposure with respect to the effects on newborn neurobehavioral development.

Methods: We conducted a data analysis of de-identified pilot cohort data using multiple linear regression to evaluate the effect of DDE exposure during individual trimesters of pregnancy and overall on newborn neurological development using the Neonatal Behavioral Assessment Scale (NBAS) among 52 Thai agriculture workers.

Results: In our study of an agricultural based cohort of Thai women recruited during the first trimester, we determined prenatal exposure to DDE has an effect on NBAS performance in the motor and regulation of state assessment clusters. Using multiple linear regression, we observed a significant association between decreased regulation of state average score and increased DDE exposure at all collection points during the study, where the most significant exposure was during the first trimester ($p= 0.003$, $r^2=0.295$). These results agree with current research which suggests a key period of susceptibility to pesticide toxicity in the first trimester of pregnancy. No associations between the models with the motor cluster average score as the outcome were significant.

Conclusions: There is evidence that prenatal exposure to DDE negatively affects neurobehavioral activity in neonates. Additional research is needed on a larger sample size in order to accommodate some of the limitations of the current study, and provide more power for adding additional covariates of interest to the initial full model.

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1 Introduction

1.1 Organochlorine (OC) Pesticide Use: Background and Toxicity

Organochlorine (OC) pesticides have been widely used to protect agriculture products and the population from vector borne diseases. Examples of OC pesticides include DDT (dichloro-diphenyl-trichloroethane), dieldrin, mirex, lindane and toxaphene. OC pesticides are fat soluble, and readily accumulate in fatty foods. Exposure can occur via direct contact with the chemical (i.e., in agriculture settings) or more commonly via consumption of contaminated foods like fish and dairy products (1). The compounds can travel via wind and water, and as a result use in one geographical area or specific country can affect a population and environment in other places with restricted or prohibited use. In addition to acute effects from direct exposure, exposure to organochlorine compounds are associated with long-term problems with the reproductive system, immune system and central nervous system. Due to these characteristics, there is concern for long-term health effects of OC pesticides and their metabolites, as well as exposure risks due to persistence in the environment (2). Long range transport of chemicals and contaminated agriculture products, as well as biomagnification in the food chain makes existing pollution due to organochlorine pesticides and DDT in particular an important public health problem for all populations (1, 3).

DDT breaks down to its daughter compound, dichloro-diphenyl-dichloroethylene (DDE), as well as dichloro-diphenyl-dichloroethane (DDD) (Figure 1). Since DDE and DDD are degradations and metabolic products of DDT,

often exposure to these compounds happens simultaneously (4). DDE exists in the environment because of photochemical decomposition, aerobic degradation or abiotic dehydrochlorination of DDT. Of the DDE isomers, p, p DDE, is more biologically active than the o, p DDE isomer. The p, p isomer for DDT and its metabolites is also the most common isomer detected in humans and the environment. DDE undergoes a slow process of degradation, as its half-life is 5.7 years. Additionally, in environments with a history of DDT treatment, DDE has been detected in soil for up to 20 years (5).

DDT was first synthesized in 1874, and began being widely used as a pesticide in 1939 (6). DDT was widely used during World War II as an inexpensive, effective method to reduce malaria transmission, and it is still used today for this purpose in some countries. Initial reliance on DDT was also due to its ability to last a long time in the environment. This same characteristic of persistence that many people viewed as advantageous also served as a motivating factor for prohibiting DDT use (6). While some countries like the United States began to discontinue the use of DDT beginning in the 1970's, an international movement to restrict DDT use did not begin until decades later (7). In 2001, the international community finalized The Stockholm Convention, a legally binding agreement where countries agreed to reduce or eliminate the use of 12 specific persistent organic pollutants (POP's), including DDT, which cause harmful effects on human health and the environment (Figure 2). The Stockholm Convention established a worldwide precedent for reducing the use of these harmful chemicals (8, 9). Thailand signed the agreement in 2005 (10). To date,

over 150 countries have signed the convention, however many developing countries with endemic malaria still regularly use DDT for vector control. (11)

Our analysis examines the effect of DDT and it metabolites in the pilot study of Asian Women and Offspring's Development and Environmental Exposures (SAWASSDEE). The investigators set out to collect information on exposure to DDE, DDT, and DDD and the effects of exposure on neurobehavioral development in neonates among pregnant agricultural workers in Thailand. The current analysis using data from the pilot study will only consider the effects of p, p DDE prenatal exposure, because this was the only compound measured in the pilot birth cohort where a majority of the participants had compound concentrations above the limit of detection (LOD).

In Thailand, continued exposure to organochlorine pesticides, especially DDT, DDE and DDD, is a persistent issue. Over the past decade, Thailand has seen a 4 fold increase in the overall use of pesticides (12). This increase makes it more difficult for the Thai government to manage regulation of the legal pesticides in use while also identifying the use of non-legal pesticides like DDT. Although the agriculture industry makes up less than 10% of the total GDP in Thailand, 41.9% of the workforce is in the agriculture industry. Furthermore, 39% of employed females in Thailand work in agriculture.

1.2 Prenatal DDE Exposure and Neurobehavioral Development

In addition to acute and chronic health effects of DDE exposure in the general public, exposure of pregnant women is of particular concern, since the

developing fetus are known to be more susceptible to harmful effects of toxic chemicals. Many studies have reported the ability of DDE and DDT to cross the placenta, exist in breast milk, and the potential for prenatal exposure to affect neurodevelopment in the fetus and in early stages of life (13-18). Furthermore, previous studies show that as DDT breaks down, the DDE metabolite is the most abundant chemical detected in trans-placental studies and thus will provide a sensitive analysis of DDT pesticide exposure**.** (16, 19-21). Due to the mechanisms by which DDE affects the central nervous system, exposure to DDT and its metabolites in utero is of particular interest with respect to neurologic development and neurobehavioral activity. Existing research evaluating the association between prenatal DDE exposure and neurobehavioral development have presented mixed results, with most studies reporting an inverse association between exposure and developmental outcomes or no significant association between outcome and exposure.

1.2. a DDE and NBAS

Many psychological and behavioral tests exist to evaluate neurological development throughout infancy and childhood. The few studies which have investigated the association between DDE and neurobehavioral development in neonates less than two months old with the Neonatal Behavioral Assessment Scale (NBAS), have had conflicting results. It is important to note that none of these studies deal with a population that involves direct pesticide exposure like our cohort. Some studies have found an association between higher prenatal DDE exposure and neurobehavioral outcomes such as hyporflexia and decrease

in attention (22, 23) using the NBAS. However, in those studies that did find a significant association between DDE exposure and the outcome the effects were different across the behavioral categories used in the assessment (23).

Two studies found no association between prenatal DDE exposure and NBAS scores (24, 25) . Similarly, results from another US cohort did not find significant association between DDE/DDT exposure and poor scores in infants, but did find poor behavioral scores with same cohort later in life (25, 26).

1.2. b DDE and other behavioral measures during infancy

Additional studies have examined the effect of prenatal DDE exposure on neurobehavioral outcomes in infancy using different outcome measurement tools other than NBAS. A popular assessment used to evaluate organochlorine exposure and behavioral outcomes for infants 1-42 months is the Bayley scales of infant neurodevelopment (BSID) (17, 26-30). Using this assessment at 14 months of age, Forns, et al. found no significant association of prenatal DDE and neuropsychological development using the BSID (31). An assessment using the BSID scales in a Spanish cohort also showed no association between prenatal exposure to DDE and psychomotor or mental development indicators. (32). Using parent reporting, Hoyer, et al. found no significant associations between exposure and. developmental milestones crawling, sanding up and walking in infants (33).

A longitudinal Mexican cohort examined the effects of residential prenatal p, p DDE exposure on infant neurodevelopment and concluded that the “1st

trimester may be a critical exposure window to DDE, and that the target area for deficits is the psychomotor development" (30). This study was the only one prior to our birth cohort (SAWASDEE) that measured repeated measures during pregnancy.

1.2. c DDE in later childhood development

Research investigating the effects of prenatal DDT/DDE exposure on outcomes later in childhood and have presented mixed results as well. Studies investigating the association between prenatal DDE and memory, learning capacity, behavior, scholastic achievement have used assessments such as the McCarthy Scales of Children's Abilities, Strengths and Difficulties Questionnaire and Attention Deficit Hyperactivity Disorder Test (34-37). Follow up studies which first examined exposure with respect to NBAS scores found a higher risk for behaviors associated with ADHD at higher levels of p,p DDE, and weak associations between higher exposure and WISC-III outcomes (38).

The difference in outcome measurement tools, level and type of maternal exposure (i.e. through food vs. through direct contact with pesticides), time of testing and multiple data collection points vs. single observations makes the interpretation and synthesis of existing results difficult to summarize.

1.3 Neonatal Behavioral Assessment Scale (NBAS)

Our study will use the Neonatal Behavioral Assessment Scale (NBAS) (39) to investigate the relationship between prenatal exposure to DDT/DDE/DDD and

neurodevelopment. The NBAS became available for neonatal evaluation in 1973. The most recent revision of the NBAS manual was in 2011. The NBAS is an assessment tool for neonatal neurodevelopment that has been used to investigate many different prenatal exposures, including recreational drug use(40-44) smoking (45),maternal medications (46)as well as other environmental toxins and pollutants (23-25, 47-51).

The design of the NBAS testing components are designed to account for the influence of genetic make-up/biology as well as the uterine environment in shaping the health and behavior of the newborn; also acknowledging that due to a unique environment before birth, each individual newborn will respond differently to the NBAS. The overall goal of the NBAS is to “identify and describe the individual differences in neonatal behavioral adaptation"; based on the assumptions that the newborn is a social being and competent in responding to stimuli (39). Based on this philosophy and evaluation design, the NBAS is an ideal tool for examination of the relationship between in utero environmental exposure and outcomes in the neonate. When used in a longitudinal setting, previous studies have shown that reduced performance on NBAS assessment early in infancy can predict deficiencies in neurologic functioning later in life. (52, 53)

The NBAS takes 20-30 minutes to administer by a trained evaluator and is structured to fully evaluate the behavioral capacity of newborn infants. The assessment is valid from birth to two months of age; however, the optimal time for use is in the first few days of life.

1.4 SAWASDEE Pilot Birth Cohort

Few studies have focused on pesticide exposure in the Thai population, and only two pilot birth cohort studies have been conducted to examine prenatal pesticide exposure in the Asian region. The Mahidol Study conducted in northeastern, lower north and western Thailand previously examined prenatal organochloride exposure in agriculture workers(54). Our current study, the Study of Asian Women and their offSpring's Development and Environmental Exposures (SAWASDEE) pilot study, was conducted in Fang District, Chiang Mai Province, in northern Thailand. The SAWASDEE pilot study is the first birth cohort study in the region to utilize exposure data collected multiple times throughout the pregnancy.

In an evaluation of the attitudes about pesticides in the Fang District of Thailand, women who worked in agriculture or applied pesticides before becoming pregnant, or who had a previous child were significantly ($p < 0.05$) more likely to engage in unsafe behaviors in the home during their current pregnancy"(18), compared to Thai people who did not work in the agriculture industry. This indicates that the population of interest may be at much greater risk for exposure than those not working in the agriculture industry. In previous analysis of the SAWSDEE pilot cohort, maternal blood and urine biomarkers for organochlorine pesticides were correlated with birth weight, length and head circumference in neonates, after stratification for PON1 phenotype (55).

Specifically, there are gaps in the literature studying populations where DDE/DDT chemicals may be still regularly used, and where there is little education about the potential harms of these chemicals. In this way, the

SAWASDEE pilot cohort, as well as subsequent phases of the study, have the potential to provide robust information regarding agriculture-related pesticide exposure among pregnant women, which can be used to inform use and guidelines in Thailand as well as other countries with little pesticide knowledge and a high proportion of the workforce in the agriculture industry.

Because the use of insecticide and pesticide use in most agriculture industries varies throughout the year, the exposure levels during each trimester of pregnancy and at delivery are likely to vary. Furthermore, as the fetus develops, there is opportunity for exposure levels at different times in the pregnancy to reflect changes in neurodevelopment to differing degrees. As a result, it is important to take into account all of the measurements collected throughout pregnancy. Few studies have considered more than one time point in their analysis of DDE pesticide exposure in utero. By analyzing multiple specimens over the course of the pregnancy, this study will fill gaps in the existing literature which call for improved information about the stability of the exposure to DDT and it metabolites, and offer the opportunity to identify a more specific window of exposure that could affect the fetus (56).

2 Materials and Methods

2.1 Research Goals and Hypotheses

For this paper, the specific research goals are as follows:

- Examine the extent of organochloride (OC) pesticide exposure during pregnancy in the SAWASDEE pilot birth cohort.
- Assess the extent to which blood concentrations of DDE at different times during pregnancy and overall contributes to decreased Neonatal Behavioral Assessment Scale (NBAS) performance in neonates, specifically among agriculture workers in Thailand.

Hypotheses:

2. 1.a Increased prenatal exposure to OC's measured by DDE during fetal development will result in decreased neurobehavioral scores on the NBAS.

2.1.b Prenatal blood concentration of DDE will vary over the three specimen collection times, establishing the importance of measuring OC exposure multiple times and accounting for multiple correlated biomarker measurements in the analysis.

2.2 Study Participants: SAWASDEE Pilot Cohort, Thailand

The SAWASDEE study is a longitudinal birth cohort comprised of pregnant women working in agriculture and their neonates. The goal of the study

is to understand the overall effects of prenatal environmental exposures on neurodevelopment in this population. The current study will focus on the pilot birth cohort of the SAWASDEE study, where all participants work in the Chiang Mai Province of northern Thailand. Pregnant women (n=59) who had their first prenatal clinic visit at Fang Hospital (Northern Thailand) were recruited into the pilot cohort. Recruitment for the longitudinal study was conducted from March 2011 to February 2012. In order to be eligible for the study, the pregnant women were required to fit the following inclusion criteria (55, 57):

1. aged 18-40 years
2. pregnant at enrollment and in the first trimester or early second trimester (16 weeks' gestation or less)
3. occupation is farmworker
4. no serious medical problems in the mother such as hypertension, diabetes, thyroid disease, HIV
5. Possession of Thai identification card permitting hospital and antenatal clinic access with at least one monthly prenatal visit allowed
6. Spoke Thai as primary language at home
7. Current residence in their Fang district for at least 6 months including planned residence at least 1 month after delivery

This pilot birth cohort was followed longitudinally through the pregnancy to the first 3 days post-delivery. All study protocols were reviewed by the Ethic Boards of Chiang Mai University, the Thai Ministry of Health and the Institutional Revie board of Emory University.

2.3 Demographic Data Collection

Exact time of enrollment for each participant differed, however at enrollment all women in the pilot study were pregnant and at 16 weeks' gestation or earlier in their pregnancy. Specimen collection during the first trimester is a novel component unique to this study.

A structured survey which included questions about demographic information, pesticide activity, occupational information, maternal lifestyle characteristics and medical history was given to each participant at the time of enrollment, 28 weeks' gestation and 36 weeks' gestation. Additional birth characteristics were obtained from birth records.

2.4 Exposure Assessment: DDE in Maternal and Cord Blood

Maternal blood was collected three times during pregnancy: at enrollment (M1); 32 weeks of pregnancy (M2); and, at delivery (M3). At the time of birth, an additional blood sample (C) was taken from the umbilical cord vein. Maternal whole blood and whole cord blood was centrifuged and plasma was separated form red blood cells for analysis. Full specimen data collection has been described in previous publications on this cohort (55).

Maternal blood from each of the three collection time points during pregnancy and infant cord blood were analyzed for 4 specific isomers of organochloride compounds: p,p, DDT, o, p, DDT, p, p, DDD , p, p, DDE, and p, p, DDT. For this study, the outcome of interest is p, p DDE. DDE concentration

was measured in plasma ng/mL. All exposure distributions for DDE measured at 4 time points (C, M1, M2 and M3) were right skewed and so were $\log_{10}$ transformed before being used in the analysis.

2.5 Outcome Assessment: NBAS Performance

The NBAS was administered to 52 infants 0-3 days after birth. The assessment is comprised of 28 behavioral components, scored on a 9 point scale, and 18 reflex components, scored on a 4 point scale. While an item-by-item analysis is an option, the most commonly used approach in analyzing results of the NBAS and drawing conclusions for research is to utilize the seven cluster scoring system developed using factor analysis and based on the natural organization of behavior and skill development in newborns (58). The seven clusters each contain three to six testing items and are labeled as habituation, orientation, motor performance, range of state, regulation of state, autonomic stability, and abnormal reflexes(39) (Figure A.3). This analysis focuses on two clusters, motor and regulation of state. The clusters under investigation were chosen based on results from past studies (23). To obtain a cluster score, the testing items within a given cluster were averaged. The NBAS is administered by a trained health professional in order to account for as much inter-rater variability as possible. For our study, a trained nurse conducted all assessments at or before 3 days after birth.

2.6 Statistical Analysis

Statistical analysis was conducted using SAS software, version 9.3 (SAS Institute, Inc., Cary, North Carolina). Univariate analyses were conducted as a descriptive procedure in order to assess the skewness of all exposure and outcome variables. The exposure at all four collection points as well as the average DDE measure were right skewed, so the exposure data was $\log_{10}$ transformed as this was a better fit for the exposure data compared to the crude measurements. Additionally, univariate analyses were conducted using covariates and other descriptive variables in order to describe the study population (Table 1).

Separate multilinear regression models were fit for the 2 NBAS clusters of interest for each of the four specimen collection points, as well as for the average DDE concentration. The average score of the individual testing items in each cluster were treated as a continuous outcome. DDE concentration at enrollment (M1), 32 week's gestation (M2) and at delivery were included in the regression models as independent predictors of the outcome, along with the covariates of interest. Since the NBAS does not have a set clinical cut point for normal range for the clusters under investigation, logistic regression was considered but not included in the final results. The outcomes were evaluated for normality, Based on graphically distribution, and measures of skewness and kurtosis within the distribution of the variable. This is also reflected in Table 1, The models were assessed for confounding using the change in estimate approach, where removal of any covariate or set of covariates from the model which resulted in less than

10% of a change in estimate from the full model would be considered as a final model.

Covariates were initially selected based on the characteristics associated with NBAS scores or DDE exposure in the literature. These covariates included in the full model include maternal age, household income, maternal education and maternal ethnicity. Due to a small sample size, sparse data in some categories, and lack of heterogeneity in the distribution of the covariates across participants, the measurement of household income and maternal education was dichotomized to less than 6,000 baht or greater than 6,000 baht for income, and any vs. no education for the maternal education variable. Alcohol consumption were not included in the model selection process because all participants in this pilot cohort reported no alcohol consumption. Covariates in the full model included maternal age (continuous), maternal education status (categorical), Maternal Income (categorical), and Ethnicity (categorical). Covariates that appear in each final model had less than a 10% change in estimate compared to the full model, and were more precise than the full model.

3 Results

3.1 Demographic Characteristics

The overall rate of retention from enrollment to delivery was 56/59 participants (95%). Among those with incomplete data (n=3), two participants were lost to follow up and one participant was excluded due to a spontaneous abortion. Participants that did not have a recorded cord blood DDE measurement were not included in the analysis for this study. The final population used in the analysis includes 52 participants (Figure A.4). As previously mentioned some infants did not have measurements for all 7 clusters of the NBAS, but were still included in the analysis since the clusters are being treated as independent outcomes.

Table 1 shows the demographic characteristics of the study population (n=52). The study primarily consisted of women who had no schooling (63.5%), had low household income, were of Thai (21.2%) or Thai Yai (57.7%) ethnicity, and born in Burma (61.5%). The mean age for the population was 26.2 years at enrollment (range 18-35 years), and all participants were either married (9.6%) or living as married (90.4%). The mean gestational age at birth was 38.2 weeks (range 35-41 weeks). 6 (11.5%) infants in the study were born before 37 weeks, and thus considered late preterm births. Most women were enrolled during their first trimester (75%).

3.2 Exposure Distribution

Table 2 shows the crude distribution of p, p DDE concentrations in maternal blood during pregnancy. Dude to the skewness of DDE measurements for all time points, the data were $\log_{10}$ transformed and the transformed values were used in the final analysis (**Table 3**). 3 maternal blood samples (at time of enrollment, at 32 weeks' gestation and at delivery) were collected for each participant along with a blood sample collected from umbilical cord blood at the time of delivery. DDE concentration in maternal blood appears to remain consistent throughout the pregnancy, with a slight increase in the average DDE concentration at the 32-week collection time for maternal blood (M2). Average DDE concentration in fetal cord blood was less than average maternal concentration at any time point.

3.3 Outcome Distribution

We determined that average summary cores for the range of state cluster and the motor cluster were normally distributed. The motor cluster follows an approximately symmetric distribution (skewness = 0.15, kurtosis=-0.42). The distribution of the regulation of state outcome was less normal with a moderately symmetric distribution (skewness=2.49) and a leptokurtic distribution (kurtosis=8.57). When we look more closely at the summary scores for the regulation of state cluster, it is evident that these summary statistics are strongly influenced by one outlier, which notably changes the skewness and kurtosis due to the small sample size of this study. When this single outlier is removed, the

range for this outcome is more normally distributed (skewness=1.53, kurtosis= 3.47). **Table 4** shows the distribution of all 7 clusters measured in the NBAS assessment, reported as average values and sum values. Based on what existing literature identifies as significant or borderline significant ($p<0.05$) in the examination of neonatal neurobehavior, our outcomes of interest were the motor cluster and the regulation of state cluster. In accordance with other studies, we utilized the average score across testing items for each infant as our outcome measure for each regression model. For linear regression analysis, motor cluster scores as well as regulation of state cluster scores were treated as continuous outcome variables. Both clusters of interest exhibited a narrow range in comparison to other clusters included in the assessment. In comparing the summary statistics of each cluster, it is important to note that all clusters incorporate a different number of individual scoring items and so have different maximum scores (**Figure 3**).

3.4 Linear Regression Models

The results of linear regression for the motor and regulation of state cluster for the average DDE across study period, as well as each individual specimen collection point are presented with regression coefficients, 95% confidence intervals and corresponding p-values for the motor cluster and regulation of state cluster in **Table 5.** Final models for regulation of state and each of the specimen collection points were significant. We observed a significant association between decreased regulation of state average score and increased

average DDE Exposure (p= 0.004, r^2 =0.287), Exposure measured at enrollment (p= 0.003, r^2 =0.295), and exposure measured at 32 weeks' gestation (p= 0.004, r^2 =0.286) when controlling for maternal age. Additionally, we observed a significant negative association between regulation of state score and DDE exposure at delivery when controlling for maternal age (p= 0.004, r^2 =0.255), and for exposure measured in cord blood when considering the full model (p= 0.011, r^2 =0.286). No associations between the models with the motor cluster average score as the outcome were significant.

Results which examined the relationship between average DDE exposure and motor cluster scores on NBAS, the covariate which had the largest influence on the outcome was maternal education (Table 6) , although all partial correlation coefficients for this relationship were very small and not significant at $p<0.05$.

Partial correlation results for the regulation of state clusters showed that in the final model not including maternal age as a predictor, overall the covariates were more influential than for the motor cluster (Table 7). With respect to regulation of state, Maternal Education was once again the strongest predictor, followed by household income level.

4 Discussion and Study Limitations

Four studies have evaluated the effect of prenatal DDE exposure and neurobehavioral performance using the NBAS (23-26). In these studies, only one measure of prenatal exposure was collected, so the authors were not able to assess the effects of the exposure over time, and how exposure levels at different time in the pregnancy may affect the outcome. Our study collected four blood samples from time to enrollment in the study until delivery. Using repeated exposure data, we independently examined the effects of DDE levels in each trimester on results of the NBAS.

The results of our analysis indicate an association between prenatal DDE exposure and adverse neurobehavioral effects in neonates, specifically in the area of state regulation. Items on the NBAS scoring system included in the Regulation of State cluster include consolability and self-quieting. This cluster deals mostly with the ability of the infant to move between states of sleep and alertness without stress and without needing much support from a caretaker (39) With respect to state regulation, we observed similar patterns as past studies, although only one other concentration levels in the SAWASDEE cohort were higher among the population on average.

We observed a relatively constant average DDE concentration in maternal blood across samples, followed by a marked decrease in DDE measured in cord blood. Organochlorine compounds have a longer half-life than other common

pesticides such as organophosphates, and this likely contributed to the reduced variability in concentration over the short study period, on average (56). As a result, the characteristics of OC compounds might this have less of an effect over such a relatively short period using measurements during a pregnancy, especially compared to organophosphates.

This study was a preliminary analysis in preparation for a large cohort study with the SAWASDEE population. Due to small sample size in the pilot birth cohort, there are many limitations to this analysis. The repeated measure collected for each participant as well as useful information on participant lifestyle and demographics lend themselves well to a longitudinal analysis approach. We attempted to fit the full model of interest (4 exposures in addition to maternal age, education, household income and ethnicity) using a correlation structure and random intercept for participant, however all models and correlation structures run did not coverage and so estimate of effect was not possible using this method. It is likely that our small sample size contributed to the non-convergence, and as the study moves forward with the main cohort (n=300) this approach should be revisited.

Other papers have included maternal IQ as an important covariate that could be a potential confounder. Our pilot study did not collect this information and so we were not able to include this factor in the analysis. Furthermore, since all participants in the pilot cohort reported no alcohol or drug use, we are unable to assess the degree to which this may affect the overall estimate of effect in this population.

We attempted to also take breastmilk concentrations into account for this study, however the participants in this study population had levels of pesticide concentration that were below the limit of detection. Further study on this population should investigate this component further.

5 Conclusions and Future Directions

In our study of an agricultural based cohort of Thai women recruited during the first trimester, we determined prenatal exposure to DDE has an effect on NBAS performance. Furthermore, exposure affects the motor and regulation of state neurobehavioral assessment clusters differently. Using multiple linear regression, we observed a significant association between decreased regulation of state average score and increased DDE exposure at all collection points during the study, where the most significant exposure was during the first trimester ($p= 0.003$, $r^2 =0.295$). These results do not agree with current research, which suggests a key period of susceptibility to pesticide toxicity in the first trimester of pregnancy, as shown by the summary results for each window of measurement during the study as well as the average, and is likely due to the correlation between repeated measurements over thecourse of pregnancy. No associations between the models with the motor cluster average score as the outcome were significant. Additional research is needed to explore the relationship between organochlorine pesticide exposure and neonatal behavioral development to further investigate the exact biological pathways that cause neurobehavioral deficiencies.

The next steps for this study are to expand the current analysis on the pilot birth cohort and to all seven NBAS clusters across DDE measurements. In addition, the methods for correlated data analysis should be revisited in the larger main birth cohort. For the main cohort study using this population, we begin recruitment in May 2017 and hope to enroll a minimum of 300 pregnant

women in the study. The study will examine acute and chronic effects of pesticides and will involve two different study cites in Thailand, including the site examined in our study. A larger sample size will be able to accommodate some of the limitations of the current study, and provide more power for adding additional covariates of interest to the initial full model. It would be particularly interesting to see if any correlation structures ran on a larger sample size from this cohort.

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Tables and Figures

Table 1: Pilot Birth Cohort Characteristics

(n=52)

Characteristic	n	%
Household Income		
1500 Baht or less	1	2
1501-3000 Baht	8	16.3
3001-6000 Baht	24	49
6001-9000 Baht	10	20.4
9001-12000 Baht	3	6.1
12000 + Baht	3	6.1
Missing	3	6.1
*note: 1000 Baht is the equivalent of about $30 USD		
Maternal Education (highest level attended)		
Never attended school	33	63.5
Primary School	9	17.3
Junior High/High School	2	3.9
High School - No Diploma	7	13.5
Some College	1	1.9
Ethnicity		
Thai	11	21.2
Thai Yai	30	57.7
Chinese	2	3.9
Other	9	17.3
Marriage Status		
Married	5	9.6

Living as Married	47	90.4
Preterm Birth		
Late Preterm Birth	6	11.5
Full Term Birth (37 weeks or more)	46	88.5
Plurality		
0	21	40.4
1	21	40.4
2	6	11.5
3	4	7.7
Maternal Country of Birth		
Thailand	19	36.5
Burma	32	61.5
Other	1	1.9
Trimester of Enrollment		
1	13	25
2	39	75
Season Of Enrollment		
Dry	9	17.31
Hot	11	21.15
Rainy	32	61.54

Table 2 : Summary Statistics for Organochloride Concentration in Blood (Crude), Plasma ng/mL

Collection Time	Biomarker	Specimen Type	n	Mean	Median	SD	Range	IQR
M1	p, p, DDE	Maternal Blood	52	2.427	1.499	2.678	11.342	2.365
M2	p, p, DDE	Maternal Blood	52	2.799	1.555	3.259	15.547	3.265
M3	p, p, DDE	Maternal Blood	52	2.602	1.312	3.764	24.120	2.626
C	p, p, DDE	Cord Blood	52	0.844	0.468	1.194	6.954	0.649
Average	p, p, DDE	Both	52	2.168	1.211	2.511	13.939	2.315

**Crude measures of DDE were consistently skewed to the right for all time points. As a result, log base 10 transformations were calculated and used in the final analysis.

Table 3: Summary Statistics for Organochloride Concentration in Blood (Log 10 Transformed), Plasma ng/mL

Collection Time	Biomarker	Specimen Type	n	Mean	Median	SD	Range	IQR
M1	p, p, DDE	Maternal Blood	52	0.14	0.18	0.48	1.95	0.70
M2	p, p, DDE	Maternal Blood	52	0.19	0.19	0.50	2.08	0.78
M3	p, p, DDE	Maternal Blood	52	0.15	0.12	0.48	2.18	0.66
C	p, p, DDE	Cord Blood	52	-0.31	-0.33	0.44	2.15	0.54
Average	p, p, DDE	Both	52	0.11	0.08	0.45	2.00	0.70

Table 4: Summary Statistics for NBAS Outcomes

Cluster (Average Score)	n	Mean	Median	SD	Range	IQR
Habituation	52	7.56	7.5	0.3	1	0.38
Orientation	52	6.24	6.43	0.84	3.57	0.86
Motor	52	6.04	5.8	0.72	3.2	1
Range of State	52	4.1	4	0.68	4	0.5
Regulation of State	52	3.98	3.75	0.71	4.25	0.75
Autonomic Stability	52	6.02	6.3	0.73	3.3	0.55
Abnormal Reflexes	52	1.65	1.61	0.07	0.44	0.06

*Note there are two main ways to report NBAS outcomes; The most common method for reporting is reporting cluster averages, and alternatively Lester (1982) recommends using the sum scores of all the items in each section

** For this analysis, we only analyzed two of the 7 NBAS clusters: regulation of state and motor cluster, and these are highlighted in the table.

Table 5: Linear Regression Results: Final Model

Regulation of State Cluster	n	β	95%CI	Final Model++	p-value	R-sq
log(10) Average DDE	52	-0.12	(-0.599, 0.369)	DROP Age	0.004+	0.287
log(10) M1 - Maternal Sample at Enrollment*	52	-0.2	(-0.651, 0.255)	DROP Age	0.003+	0.295
log(10) M2 - Maternal Sample at 32 Weeks Gestation	52	-0.1	(-0.524, 0.334)	DROP Age	0.004+	0.286
log(10) M3 - Maternal Sample at Delivery	52	0.009	(-0.395, 0.414)	DROP AGE, EthCode	0.004+	0.255
log(10)C - Cord Blood Sample at Delivery	52	0.007	(-0.4741, 0.4887)	Full Model**	0.011+	0.286
Motor Cluster ^	n	β	95%CI	Final Model	p-value	R-sq
log(10) Average DDE	52	0.066	(-0.506, 0.638)	Full Model**	0.959	0.023
log(10) M1 - Maternal Sample at Enrollment*	52	0.172	(0.324, 0.669)	DROP AGE, EthCode	0.769	0.025
log(10) M2 - Maternal Sample at 32 Weeks Gestation	52	0.002	(-0.051, 0.508)	Full Model**	0.964	0.022
log(10) M3 - Maternal Sample at Delivery	52	0.148	(-0.354, 0.650)	DROP EthCode	0.873	0.027
log(10)C - Cord Blood Sample at Delivery	52	0.023	(-0.538, 0.538)	Full Model**	0.963	0.022

*women in the study were enrolled prior to 16 weeks' gestation (mean= 12.8 weeks, IQR= 10-16 weeks)

**full model= exposure+ age, education, income, ethcode

^for the motor cluster, all crude and full models, as well as those run to assess confounding by change in estimate approach were not significant

+significant result ($p<0.05$)

++ for the final models, a covariate was not included in the final mode if by excluding it from the model it did not change the estimate of the regression coefficient by more than 10%, compared to the full model

Table 6: Motor Cluster Models with Partial Correlation Coefficients
Full Model:

$$AMOTOR = \beta_0 + \beta_1(AvgDDE) + \beta_2(Ethnicity) + \beta_3(Educ) + \beta_4(Income) + \beta_5(Age) + e$$

Variable	n	β	SE	p-value	Partial Correlation Coefficient (Type I)
intercept	52	6.215	0.908	<.0001	NA
Log(10) Average DDE	52	0.066	0.284	0.817	0.008
Ethnicity	52	0.027	0.112	0.810	0.001
Maternal Education	52	0.080	0.126	0.528	0.009
Household Income	52	-0.026	0.144	0.858	0.001
Maternal Age	52	-0.010	0.023	0.673	0.004

**NOTEL: for the motor cluster, the full model was also the best fit model

Where:

AMOTOR = Average motor cluster score across testing items

AvgDDE = $\log_{10}$ transformation of average DDE concentration (ng/Ml) during pregnancy

Ethnicity = Maternal ethnicity

Educ = Maternal education

Income= Household income, in Baht

Age = maternal age at study entry

Table 7: Regulation of State Models with Partial Correlation Coefficients
Full Model:

$$AREGOFSTATE = \beta_0 + \beta_1(AvgDDE) + \beta_2(Ethnicity) + \beta_3(Educ) + \beta_4(Income) + \beta_5(Age) + e$$

Variable	n	β	SE	p-value	Correlation Coeff.
intercept	52	2.190	0.777	0.007	NA
Log(10) Average DDE	52	-0.121	0.243	0.621	0.029
Ethnicity	52	0.137	0.096	0.159	0.023
Maternal Education	52	0.166	0.108	0.130	0.147
Household Income	52	0.297	0.123	0.020	0.118
Maternal Age	52	0.009	0.020	0.649	0.005

Final Model (Drop Age):

$$AMOTOR = \beta_0 + \beta_1(AvgDDE) + \beta_2(Ethnicity) + \beta_3(Educ) + \beta_4(Income) + e$$

Variable	n	β	SE	p-value	Partial Correlation Coeff.
intercept		2.453	0.518	<.0001	NA
Log(10) Average DDE		-0.115	0.240	0.633	0.029
Ethnicity		0.130	0.094	0.172	0.023
Maternal Education		0.164	0.107	0.130	0.147
Household Income		0.296	0.122	0.019	0.118

Where:

AMOTOR = Average motor cluster score across testing items

AvgDDE = $\log_{10}$ transformation of average DDE concentration (ng/Ml) during pregnancy

Ethnicity = Maternal ethnicity

Educ = Maternal education

Income= Household income, in Baht

Age = maternal age at study entry

Figures

Figure 1*: Breakdown of DDT to DDE and DDD metabolites (59)*

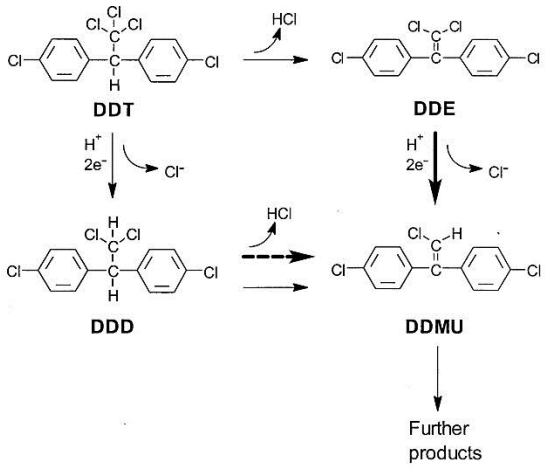
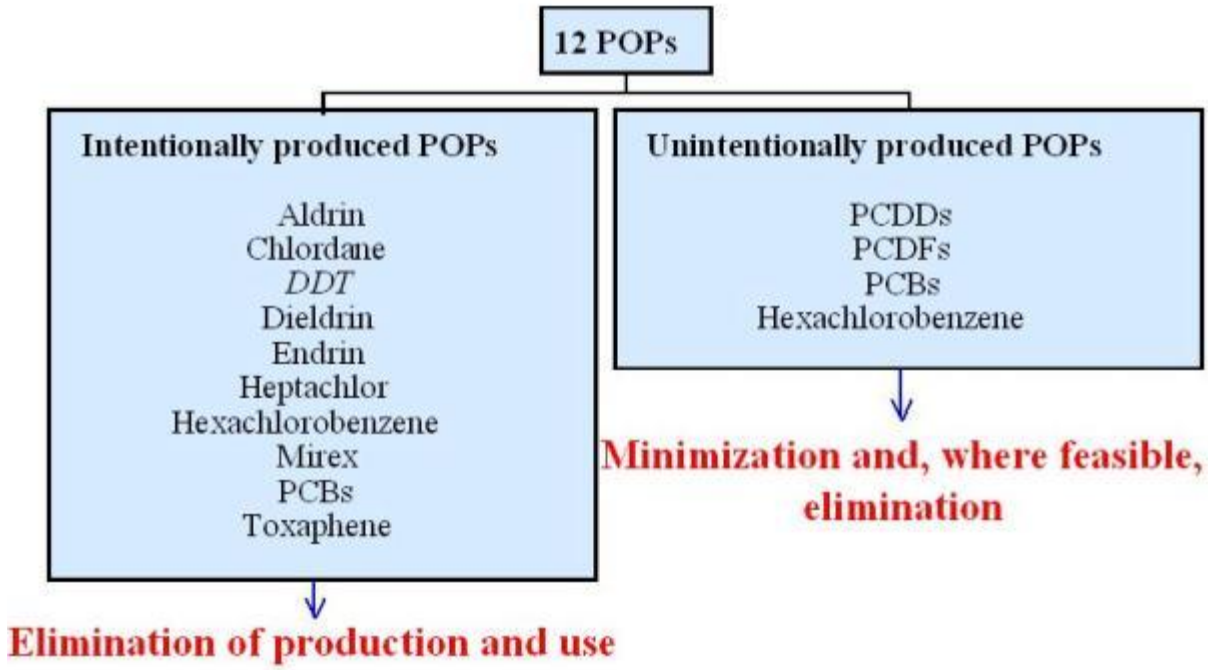


Figure 2: 12 Initial Persistent Organic Pollutants *include in the Stockholm*



Convention (2004) (60)

Figure 3 Brazleton Scoring Clusters (39)

Figure A.3: NBAS Cluster Description and Testing Items

	Measurement of Cluster Scores	Description	Items included	Max. Score
Autonomic Stability	Sum /Average		Tremors, Startles, Skin Color	27
Habituation	Sum/Average		Light, Rattle , Bell,Skin Prick	36
Range of State	Sum/Average		Peak of excitement, rapidity of build up, irritability, liability of state	36
Regulation of State	Sum /Average		Cuddliness,Consolability, Self-Quieting, Hand –to-Mouth	36
Motor	Sum /Average		Tonus, maturity, pull-to-sit, defense, activity	45
Orientation	Sum /Average		Inanimate visual, inanimate auditory, inanimate visual-auditory, animate visual, animate auditory,	63

		animate visual-auditory, alertness	
Abnormal Reflex	Count	NA	NA*

*reflex score = total number of abnormal reflexes

Figure 4 inclusion criteria for analysis