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Relationship between Pesticide Knowledge, Attitudes, and Practice (KAP) and Prenatal Organophosphate Exposure in a Pilot Thai Agricultural Birth Cohort

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Bachelor of Science

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Abstract

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Chronic exposure to OP pesticides has been linked to several negative, long-term health outcomes, including the inhibition of neurodevelopment through prenatal exposure. Due to the high risk of OP exposure among agricultural workers and the high hazard of in utero exposure, it is important to understand the factors that may affect exposure in pregnant farmers. The SAWASDEE birth cohort was established in order to assess the effect of prenatal pesticide exposure on birth outcomes in pregnant farmers working in the Chiang Mai province in Northern Thailand. The purpose of this study is to assess the relationship between pesticide knowledge, attitudes, and practice (KAP) and prenatal organophosphate exposure in this cohort. Questionnaires were administered three times through pregnancy and used to generate seven different KAP scores. Prenatal OP exposure was measured an average of eight times through pregnancy using urinary levels of dialkyl phosphate (DAP) metabolites. Using linear regression models, the relationships between each of the seven KAP scores and trimester-specific exposure measurements were assessed, controlling for potential confounding by maternal income level and parity where necessary. Usefulness score was nominally ($0.05 < p \leq 0.10$) or strongly ($p \leq .05$) significantly related to urinary DAP levels at all trimesters, where increased perceived usefulness of pesticides was associated with increased DAP levels. Responsibility score was nominally significantly related to DAP levels in the second trimester, and strongly related in the third trimester, where an increased sense of responsibility for the safe use of pesticides was associated with decreased DAP levels. There was also nominal evidence that increased KAP score is associated with decreased third trimester DAP levels, and that increased number of risky practices at home is associated with decreased total DAP levels. There were no significant relationships seen with personal susceptibility, child susceptibility, and risky behaviors at work scores. The results suggest that there is a moderate relationship between attitudes towards pesticides and OP pesticide exposure, and a possible relationship between pesticide knowledge and OP exposure. This is the first study to assess the relationship between KAP scores and OP pesticide exposure through pregnancy.

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BACKGROUND AND SIGNIFICANCE

As a nation whose economy relies primarily on agricultural exports, Thailand uses pesticides heavily to protect crops and increase crop yields. Since 2000, pesticide use in Thailand has increased more than 400% and pesticide use per unit area is the third highest among East Asian countries due to increased pest resistance and growing global food needs [Panuwet, 2012]. Because of the lack of a consolidated system for pesticide management in Thailand, pesticides are often misused, leading to overuse, increased pest resistance, environmental damage, and increased human exposure. Though the Department of Agriculture does play some role in the regulation and testing of pesticides, the smuggling of illegal pesticides into Thailand and their unregulated application remains a major problem in Thailand [Panuwet, 2008].

In a study of pregnant women in Ecuador, pesticide exposure among farmers was shown to be greater than that of non-farmers, but elevated in both populations, signifying the potential for non-occupational routes of exposure [Handal, 2015]. In the Chiang Mai province of Thailand, along with other agricultural cohorts around the world, farmers were found to have had elevated urinary concentrations of harmful constituents of pesticides, like malathion, 2,4-D, and parathion [Panuwet, 2008]. In a study of school children, children of farmers had higher levels of some pesticides than children of non-farmers living in the same communities, suggesting non-occupational routes of exposure [Panuwet, 2009].

Aside from workplace exposure to pesticides, farmers and their families can also be exposed to pesticides dragged into the home from the workplace, sometimes referred to as para-occupational exposure. Pesticide handlers have been shown to have increased levels of pesticides in their vehicles and homes compared to farmers that do not handle pesticides [Curl, 2002; Lu, 2000]. Also, among those who handled pesticides, there were significantly different levels of households pesticides based on accessibility of laundry facilities, storage of work boots, frequency of hand washing, commuter vehicle use, parking location, and safety training [Fenske, 2013], exemplifying the importance of keeping the home separate from the workplace in farmworker households.

The negative effects of organophosphate pesticides are well-reviewed. The most noticeable negative effects of OP pesticide exposure are due to the inhibition of acetylcholinesterase during acute poisoning. Due to overstimulation of the autonomic nervous system during acute poisonings, symptoms could range from muscle weakness to tremors to paralysis depending on the severity of poisoning [Chowdhary, 2014]. A 1989 report by Thailand's Office of the National Environment Board reported that 36% of reported pesticide poisonings were due to suicide attempts [Thapinta, 1998] and an estimated one-third of suicides globally are carried out by self-induced pesticide poisoning [Bertolote, 2006], exemplifying the potential lethality of acute pesticide poisoning. Furthermore, high levels of OP pesticide exposure have been associated with decreased acetylcholinesterase activity, showing a relationship between OP exposure and the mechanism underlying pesticide poisoning [Jintana, 2009].

In addition to acute autonomic effects, evidence suggests that organophosphates are endocrine disrupting chemicals (EDCs). EDCs are known to negatively impact fertility, intelligence, and overall health outcomes in epidemiological studies [Mnif, 2011; Colborn, 1993; Yang, 2006]. Fetal exposure to organophosphates has been linked to neurodevelopmental deficits in children in various studies, highlighting the risks of prenatal organophosphate pesticide exposure [Gonzalez-Alzaga, 2014; Munoz-Quezada, 2013; Eskenazi, 2007]. In the SAWASDEE cohort, the same cohort used for this study, high levels of organophosphate metabolites detected pregnant mothers were associated with decreased head circumference and birthweight in their offspring [Naksen, 2015].

Knowledge, attitudes, and practice (KAP) surveys are used in a wide-range of public health topics to reveal misconstructions that may describe potential barriers to behavior change. A KAP survey for pesticide use has been used to a wide variety of agricultural settings to assess gaps in pesticide knowledge, attitudes towards pesticide usage, and safe application/handling practice. In a similar study population to the SAWASDEE birth cohort used for this study, Lorenz *et al.* (2012) used KAP surveys in a cross sectional study of 76 pregnant Thai women to assess the relationship between scores in the knowledge, attitudes, and practice sections and found slight associations between pesticide knowledge and risky practices. Studies in Uganda [Oesterlund, 2014] and Tanzania [Lekei, 2014] investigating the relationship between KAP and self-reported acute pesticide poisoning have yielded no results, with the exception of a significant association between sucking and blowing the nozzle of the pesticide sprayer and self-reported pesticide intoxication in Ugandan farmers. Quandt (2006) reviews workplace, household, and personal predictors of pesticide exposure for farmworkers. Only a third of the studies reviewed used environmental or biomarker estimates to assess exposure. These studies revealed protection from pesticide exposure with daily house cleaning, use of protective equipment, and proper handling of laundry. Despite considerable research on pesticide KAP among various farming populations, and the relationship between pesticide KAP and self-reported health outcomes, there are no studies that examine the relationship between the results of KAP questionnaires and longitudinal pesticide exposure levels in pregnant mothers.

METHODS

Study Population

All study protocols were reviewed and approved by the Institutional Review Board of Emory University and the Ethic Boards of Chiang Mai University and the Thai Ministry of Health. In a collaboration between Emory University and Chiang Mai University faculty, the SAWASDEE birth cohort was established in order to study the neurodevelopmental impacts of prenatal pesticide exposure. The SAWASDEE cohort is composed of pregnant women who work as agriculture workers in an agriculturally dense region in Northern Thailand. This is the first birth cohort to assess pesticide exposure continuously through pregnancy, allowing for trimester-specific exposure measurements and the examination of longitudinal trends in pesticide exposure.

From March 2011 to February 2012, fifty-nine women were recruited from the antenatal care unit at Fang Hospital in the Chiang Mai province. The inclusion criteria included: (1) aged 18-35 years, (2) pregnant for sixteen or less weeks at enrollment, (3) worked as a farmworker, (4) in good health, (5) planned on living in Fang District longer than six months. After three women three were loss due to follow-up because of spontaneous abortion or failure to return the hospital, the final sample size for this study was fifty-six.

KAP Measurement

KAP questionnaires were orally administered by trained nurses at Fang Hospital at baseline, at 24 gestational weeks, and on the day of delivery. The questionnaire was composed of various sections: (1) occupational information/physical exertion, (2) housing characteristics, (3) house cleaning, (4) household pesticide use, (5) pesticide knowledge, attitudes, and practices (KAP), (6) demographics, (7) medical history, (8) pregnancy history, (9) paternal demographics, (10) pregnancy history, (11) household members, (12) pets, (13) medical testing, (14) personal habits information, and (15) other exposures and concerns. The relevant portion of the questionnaire for KAP purposes, along with the system for scoring the questionnaire, is described in the supplementary material of Lorenz (2012) and described in less detail below.

Seven scores (one knowledge score, four attitude scores, two practice scores) were calculated from these questionnaires. For scores tabulated from multiple questionnaire administrations (except the practice scores), a mean score was calculated by taking the mean of the scores across questionnaires. The ‘knowledge’ score was calculated by summing the number of correct responses to questions about prenatal health, health impact (acute and long-term) of pesticides, intake pathways, protection against exposure, etc. There were 49 questions used to calculate the knowledge score, so possible knowledge scores ranged from 0–49. Responses of “not sure” or “don’t know” were considered incorrect. Knowledge scores were only calculated for the baseline and 24-week questionnaires because many of the relevant questions were not answered during the final questionnaire administration. In analysis, knowledge scores were assessed as a continuous exposure variable.

The four attitude scores were calculated from questions about perceived susceptibility to the effects of pesticides, personal responsibility for their safe use, and their usefulness at home and occupationally. The first three scores measured the participant’s perceived susceptibility to the health effects of pesticides, her attitudes regarding her child’s susceptibility to the health effects of pesticides, and, using Sam (2008) as a guideline, the participant’s acceptance of responsibility for the safe use of pesticides. For these three scores, responses indicating higher belief in susceptibility or acceptance of responsibility were awarded two points, and responses of “don’t know” or “not sure,” which indicate moderate attitude, were awarded one point, so possible scores ranged from 0–4, 0–8, and 0–12, respectively. Like the knowledge score, these attitude scores were only calculated for the baseline and 24-week questionnaires because many of the relevant questions were not answered during the final questionnaire administration. The fourth score, which measured the participants’ attitudes towards the usefulness of pesticides, both at home and workplace, was based on reasons why the participants used pesticides. This 'pesticide usefulness' score was only calculated for women who have personally applied or handled pesticides at work or at home, and was only calculated for the baseline questionnaire because of limited data from the other two questionnaires. Possible scores for this section ranged from 0–13. All attitude scores were assessed as a continuous exposure variable.

Two practice scores, one for pesticide practice at home and another for pesticide practice at work, were calculated by summing the amount of reported "risky behaviors," defined by Goldman *et al.* (2004) as behaviors known or thought to be associated with pesticide exposure. The behaviors included: improper handwashing, delayed bathing, lack of protective clothing, improper storage of clothing, low frequency of house cleaning, eating fruits and vegetables directly from the field, wearing work shoes into the house, and wearing work clothes into the house. Lorenz *et al.* (2014) added one additional risky behavior to the Goldman *et al.* (2004) list – storing pesticides in or around the home. In analysis, these scores were assessed as ordinal exposure variables.

Organophosphate exposure measurement

Prenatal exposure to organophosphate (OP) pesticides was measured by quantifying urinary dialkyl phosphate (DAP) metabolites, which are considered some of the strongest markers of OP pesticide exposure [Sudakin, 2011]. Six DAP metabolites (DMP, DMTP, DMDTP, DEP, DETP, DEDTP) could be used to calculate summed DAP levels. Diethyl-substituted DAPs (DEAP) and dimethyl-substituted DAPs (DMAP) were calculated by summing molar concentrations of DEP, DETP, DEDTP and DMP, DMTP, DMDTP, respectively. Total DAPs were calculated by summing DEAP and DMAP. ∑DAP was the summary pesticide exposure measure used for analysis in this study.

Urine samples from the mothers were collected at multiple time points throughout pregnancy, with an average of eight samples (number of measurement occasions ranged from 5 to 13) collected through pregnancy from each woman. One urine sample was collected after birth, but this measurement was not included in final analysis. Urine samples were collected at each visit to the antenatal clinic using 50ml polypropylene cup. All samples were stored in vials and frozen until analysis was carried out at Chiang Mai University. Detailed in Prapamontol *et al.* (2014), gas chromatography and flame photometric detection were used to measure levels of DAP metabolites, and the limits of detection (LOD) for these metabolites were reported from 0.1 ng/mL urine to 2.5 ng/mL urine. The relative recovery range using this method was 94.4% - 119%. All metabolite measurements were converted to their molar equivalents and summed to the summary measures described above. Measurement outcomes were averaged by trimester and $\log_{10}$ transformed for analysis because of heavily right-skewed distributions.

Analysis

Data were analyzed using SAS software, version 9.3 (SAS Institute, Inc., Cary, North Carolina). Univariate analysis was used to assess KAP scores and various exposure estimates by trimester. Correlation measures were used to compare the knowledge and attitude scores (excluding the usefulness score) across measurement occasions. Also, correlations between mean DAP metabolite levels were assessed. In assessing for potential confounding, the relationships between potential confounders (age, education level, income, and parity) and KAP scores and were assessed using simple linear regression. This was also done for the relationship between these potential confounder and DAP measurements. Season of DAP measurement was assumed to be associated with OP exposure, but showed no relationship to KAP scores, so season was not evaluated as a confounder.

To assess relationships between KAP scores and DAP levels, linear regression models were used. Potential confounders were included in linear regression models when they were associated with both the KAP score and exposure estimate. In linear regression models, all KAP scores were assessed as continuous variables in order to increase statistical power. The use of ordinal variables, such as the risky behavior scores, is not ideal, but acceptable for linear regression models [Winship, 1984]. DAP measurements were $\log_{10}$ transformed in order to account for heavy skewness in the distribution of OP exposure.

RESULTS

Descriptive Statistics

Demographic characteristics of the 56 SAWASDEE cohort participants are presented in **Table 1**. The mean age at enrollment was 26.3 years and ranged from 18 to

35. Mean gestational age at enrollment was about 13 weeks and mean gestational age at birth was 38.1 weeks (normal gestational age at birth is about 40 weeks). 7/56 (12.5%) of the cohort had a preterm birth outcome, which is defined as birth before 37 gestational weeks. All women in the study were either married or living with their significant other. A majority of the women received no education or only primary school education (82.1%) with only one woman graduating secondary school with a diploma. About two-thirds of the women live under the international poverty line of 6,000 baht per year. The women come from mixed ethnic backgrounds, but 85.7% of the women spoke some dialect of Thai at home. 35/56 (62.5%) of the mothers had at least one child. Women were enrolled during all three seasons, with 13 being enrolled during the dry season, 24 during the hot season, and 19 during the rainy season.

KAP Scores

Results from the KAP questionnaires are presented in **Table 2**. All of the knowledge and attitude scores were greater on the 24 week questionnaire than the baseline questionnaire, except the usefulness score which was only measured at baseline. Because risky behavior at work score was only measured when mothers were working, there were 40 risky behavior at work scores measured at the 24 week mark and only 9 measured at delivery. Only for working mothers, 35/52 reported at least one risky behavior at work at baseline, 17/40 reported at least one risky behavior at work at 24 weeks, and 4/9 reported at least one risky behavior at work at baseline. The number of risky behaviors at home seemed to remain consistent across measurement occasions.

When KAP scores were measured on multiple questionnaires, correlation among scores was assessed. Using a Pearson correlation coefficient, knowledge scores from the baseline and 24 week questionnaires are shown to be mildly correlated ($r = 0.32$, $p = 0.015$). Using Spearman correlation coefficients, there was mild correlation between the two personal susceptibility scores ($r = 0.36$, $p = 0.006$) and the responsibility scores ($r = 0.41$, $p = 0.002$), and strong correlation between the three risky behavior at home scores ($r = 0.52/0.59/0.46$, $p < 0.0005$). There was little evidence of correlation between the child susceptibility scores ($r = 0.22$, $p = 0.109$). Because of limited risky behavior at work scores at 24 weeks and delivery, correlation could not be assessed.

In assessing for confounding, all the relationships between all KAP scores and potential confounders (income, age, education, and parity) were assessed using simple linear regression. There was no evidence of any relationship between age and any of the KAP scores. There were strong positive relationships ($p < .05$) between education level and knowledge, responsibility, and risky behaviors at home (at baseline) scores, and between income and risky behaviors at home (at baseline) scores. There was also a strong negative relationship between education level and the usefulness scores. There were nominal positive relationships ($0.05 < p < 0.10$) between income and knowledge scores, and between parity and risky behaviors at home (at 24 weeks) scores. There were nominal negative relationships between income and risky behaviors at work (at baseline) scores, and between parity and risky behaviors at work (at 24 weeks) scores.

OP Pesticide Exposure

Mean urinary ΣDAP, ΣDEAP, and ΣDMAP levels by trimester and overall are presented in **Table 3**. Overall, metabolite levels were heavily right-skewed with wide ranges. All three summary measures decrease from the first trimester to the second to the third. 25/34 mothers with first trimester measurements had only one measurement occasion during the first trimester and the other 9 had two measurement occasions. Because of the smaller number of measurement occasions during the first trimester, DAP and DEAP levels have the largest standard deviations and widest ranges in the first trimester. In assessing for correlation between values, there is no significant correlation between metabolite levels across trimesters. There is significant correlation ΣDAP and ΣDEAP, and ΣDAP and ΣDMAP levels within trimesters, but less correlation between ΣDEAP and ΣDMAP levels within trimesters.

In assessing for confounding, all the relationships between DAP measurements (by trimester and total) and potential confounders (income, age, education, and parity) were assessed using simple linear regression. There was no evidence of any relationship between age and education, and DAP measurements. There were strong negative relationships ($p < 0.05$) between parity and DAP mean measurement in the second and third trimesters, where greater number of children was associated with decreased levels of DAP. There was also a nominal negative relationship ($0.05 < p < 0.10$) between income and mean DAP levels in the third trimester. These measures of association were consistent with those seen with analysis exploring relationships between potential confounders and DEAP levels.

Linear Regression

In testing for confounding, it was determined that maternal income level was a possible confounder in relationship between knowledge score and urinary DAP levels, and both maternal income level and parity were significant confounders in the relationship between practice scores (both at work and at home) and urinary DAP levels. All beta coefficients with associated p-values from all linear regression models are presented in **Table 4**.

There was a nominally ($0.05 < p \leq 0.10$) or strongly ($p \leq 0.05$) significant relationship between usefulness score, measured at baseline, and all trimester-specific DAP levels. For the first ($\beta = 0.101$, $p = 0.10$) and second ($\beta = 0.068$, $p = 0.06$) trimester DAP levels, there was a nominal relationship between usefulness score and DAP levels. For third trimester ($\beta = 0.079$, $p = 0.03$) and total ($\beta = 0.065$, $p = 0.04$) DAP levels, there was a strong relationship between usefulness score and DAP levels. There was a strongly significant relationship between 24 week ($\beta = -0.087$, $p = 0.03$) and mean ($\beta = -0.081$, $p = 0.05$) responsibility scores and third trimester DAP level. There was a nominally significant relationship between 24 week ($\beta = -0.058$, $p = 0.07$) and mean ($\beta = -0.062$, $p = 0.07$) responsibility scores and total DAP level, and between baseline ($\beta = -0.045$, $p = 0.10$) and mean ($\beta = -0.062$, $p = 0.10$) responsibility scores and total DAP level. There was a nominally significant relationship between baseline ($\beta = -0.27$, $p = 0.06$) and mean ($\beta = -0.036$, $p = 0.09$) knowledge scores and third trimester DAP level. Also, there was a nominally significant relationship between number of risky behaviors at baseline and total DAP level (β = -0.079 p = 0.08). There was no evidence of any significant relationship between urinary DAP levels and any personal susceptibility, child susceptibility, and risky behaviors at work score. Beta parameters were not estimated when trimester-specific DAP measurements preceded the measurement of risky behaviors.

DISCUSSION

Until this study, there have been no studies that have assessed the relationship between pesticide knowledge, attitude, and practice, and OP pesticide exposure, measured through biologic levels of pesticide metabolites, in pregnant mothers. There have been some studies that have assessed the relationship between risky practices and environmental/biomarker evidence of pesticide exposure, but most of these studies relied on single exposure measurements and were never conducted with pregnant farmer populations [Quandt, 2006]. We postulate that having multiple OP exposure measurements through pregnancy improved the quality of the data by reducing the misclassification of OP exposure that is commonly seen when using DAP metabolite levels to approximate OP exposure [Reiss, 2015]. Also, collecting an average of eight measurements per mother (up to thirteen) allowed us to evaluate pesticide exposure by trimester, which is data that is often missing in the literature looking at the negative effects of pesticide exposure.

Mean gestational age at birth was 38.1 weeks, which was almost two weeks less than expected, and 12.5% of the mothers had a birth defined as preterm, which was unexpectedly high. We believe that is due to poor estimation of conception date by mothers. If this was the case, most measurements assumed to be first trimester measurements would be second trimester measurements, and many second trimester measurements would actually be third trimester measurements, which would change trimester specific, but not total, DAP measurements. OP exposure seemed to decrease significantly from the first to the second to the third trimester, and the range of measurements also decreased substantially. This is most likely due to the fact that the burden of childbearing increased through pregnancy, leading to mothers working less and eventually not at all. This is seen in the risky behaviors at work scores, where 54 mothers were working at baseline, 40 were working at 24 gestational weeks, and only 9 were working in the last couple weeks of pregnancy.

In testing for potential confounding, a strong relationship ($p < 0.001$) between parity (number of children), and second and third trimester DAP levels (and DEP levels) was found, where a greater amount of children was associated with decreased OP exposure. This strong relationship was unexpected and should be analyzed further. There was also a significant negative relationship between income and third trimester OP exposure, which may be due to more wealthy mothers working less, improved housing characteristics, and the ability to clean home and clothing more properly. As expected, higher education was related to increased knowledge and attitude scores, and a decreased risky behavior at home score. Higher income was associated with increased knowledge score, and decreased risky behaviors at home and work scores. The relationship between income and risky behavior scores may be due to mothers having the resources to avoid risky behaviors (i.e. access to a shower, cleaning supplies, separate storage for home and work clothes).

Usefulness score, measured at baseline, had the strongest relationship to pesticide exposure of any of the KAP scores. Usefulness score was the only score with a significant relationship to first trimester exposure measurements. In all trimesters, higher perceived usefulness of pesticides at home and the workplace was associated with higher OP exposure. The strong significance of this relationship was interesting because there were less usefulness scores (n = 31) than all other scores because usefulness score was only measured when mothers personally apply pesticides, so it would be expected that the smaller sample size would prevent significant results. It is nominal that there is a relationship between perceived usefulness of pesticides and how often that individual applies them, which could explain this relationship.

In every relationship between knowledge score and trimester-specific exposure measurements, OP exposure seemed to decrease with increased knowledge. Though, this relationship was only significant for the baseline knowledge score and third trimester exposure measurement. Because all parameter estimates for knowledge scores were negative, it seems as if small sample size and a smaller number of measurement occasions in the first and second trimesters, compared to the third, were major factors leading to insignificant results. For second and third trimester DAP measurements, higher responsibility score (i.e. greater sense of responsibility for the safe use of pesticides) was significantly associated with decreased DAP exposure. It is likely that those who believe that they are responsible for using pesticides in a safe manner are less likely to engage in risky behavior, hence the decrease in OP exposure with an increased sense of responsibility towards safety. Finally, an increased number of risky behaviors at home (at baseline) was associated with a decrease in total DAP levels, even though it would be expected that increased risky behavior would be associated with increased OP exposure.

Major limitations of this study include a small sample size and substantial intra- and inter-day variability in urinary DAP levels. There is high potential of exposure misclassification when using DAP metabolite levels to estimate OP exposure because 1) preformed DAP metabolite could be ingested with food, 2) OP pesticides have a very short elimination half-life, and 3) DAP metabolites are often not specific to one pesticide [Reiss, 2015]. In addition to possible random error due to small sample size and potential issues with OP exposure estimation, season is the strongest predictor of pesticide exposure. OP exposure is greatest in the rainy season, from July to October, when pesticides are sprayed at the highest volume. This is seen in the data, where there is little to no correlation between trimester-specific DAP, DEAP, or DMAP measurements, and no linear trend in metabolite levels when measurements are assessed individually. Because mothers were enrolled into the study throughout the year, trimester-specific exposure estimates varied greatly between mothers based on season. Season of measurement was presumably a much greater determinant of DAP levels than KAP scores, so some the effects of KAP score on OP exposure may have gotten lost in the statistical noise caused by season.

Because this study measured OP exposure at multiple time points through pregnancy, future analysis could use mixed models to assess longitudinal trends in exposure with respect to pesticide knowledge, attitude, and practice. A major barrier to conducted mixed model analysis is that season, the strongest predictor of pesticide exposure, changes over the course of pregnancy. ΣDEAP and ΣDMAP measurements were available, but not included in regression models because there was no nominal reason to believe that KAP scores would differentially affect exposure to specific DAP metabolites, but future models could incorporate specific metabolites.

CONCLUSION

Even though small sample size and highly variable DAP levels were an issue for this study, this study fills a large gap in the literature by assessing factors that affect organophosphate pesticide exposure in pregnant mothers and their unborn children, those at the highest risk of the negative effects of pesticides. London (2012) states that women's exposure to pesticides is significantly underestimated in the literature and that adverse outcomes are gender-discriminatory, which is extremely true with pregnant farmers. She also states that this erroneous risk perception puts female at greater risk of exposure. Because agriculture is often viewed as a male-dominated industry, it is easy for females to go ignored. This study aims to fill in those gaps pointed out by London (2012) by not only looking at an under-researched population, but one that is at very high risk.

The significant relationship found between increased sense of responsibility for the safe use of pesticides and decreased exposure signifies that responsible farming is better for the farmer. Arcury (2002) states that control over one's environment is a key tenant of environmental justice. It is important that farmers understand the risks involved with the application of pesticides. With proper knowledge of pesticides and a sense of the risk that their application carries, farmers will have greater control over both their work and home environments. Although there are no studies that assess the efficacy of educational intervention in reducing pesticide exposure, various studies in the United States, Thailand, and India have shown that educational intervention, even by a lay advisor, results in high retention of pesticide-related knowledge and an improvement in KAP scores in subsequent follow-up visits [Arcury, 2009; Janhong, 2005; Sam, 2008]. With a growing world population, there will be many more mouths to feed, many more crops to grow, and many more tons of pesticides to be sprayed. It is imperative that we, as the consumers, protect farmers, the producers, by giving them the knowledge needed to fully understand their environment and the risks around them.

REFERENCES

Arcury, T. A., Quandt, S. A., & Russell, G. B. (2002). Pesticide safety among farmworkers: perceived risk and perceived control as factors reflecting environmental justice. *Environ Health Perspect, 110*(Suppl 2), 233.

Arcury, T. A., Marín, A., Snively, B. M., Hernández-Pelletier, M., & Quandt, S. A. (2009). Reducing farmworker residential pesticide exposure: evaluation of a lay health advisor intervention. Health promotion practice, 10(3), 447-455.

Bertolote, J. M., Fleischmann, A., Eddleston, M., & Gunnell, D. (2006). Deaths from pesticide poisoning: a global response. The British Journal of Psychiatry, 189(3), 201-203.

Chowdhary, S., Bhattacharyya, R., & Banerjee, D. (2014). Acute organophosphorus poisoning. Clin Chim Acta, 431, 66-76.

Colborn, T., vom Saal, F. S., & Soto, A. M. (1993). Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ Health Perspect, 101(5), 378.

Costa, L. G., Cole, T. B., Vitalone, A., & Furlong, C. E. (2005). Measurement of paraoxonase (PON1) status as a potential biomarker of susceptibility to organophosphate toxicity. Clin Chim Acta, 352(1), 37-47.

Curl, C. L., Fenske, R. A., Kissel, J. C., Shirai, J. H., Moate, T. F., Griffith, W., . . . Thompson, B. (2002). Evaluation of take-home organophosphorus pesticide exposure among agricultural workers and their children. Environ Health Perspect, 110(12), A787.

Eskenazi, B., Marks, A. R., Bradman, A., Harley, K., Bart, D. B., Johnson, C., . . . Jewell, N. P. (2007). Organophosphate pesticide exposure and neurodevelopment in young Mexican-American children. Environ Health Perspect, 792-798.

Fenske, R. A., Lu, C., Negrete, M., & Galvin, K. (2013). Breaking the take home pesticide exposure pathway for agricultural families: Workplace predictors of residential contamination. *Am J Ind Med, 56*(9), 1063-1071.

Gonzalez-Alzaga, B., Lacasana, M., Aguilar-Garduno, C., Rodriguez-Barranco, M., Ballester, F., Rebagliato, M., & Hernandez, A. F. (2014). A systematic review of neurodevelopmental effects of prenatal and postnatal organophosphate pesticide exposure. *Toxicol Lett, 230*(2), 104-121. doi: 10.1016/j.toxlet.2013.11.019

Goldman, L., Eskenazi, B., Bradman, A., & Jewell, N. P. (2004). Risk behaviors for pesticide exposure among pregnant women living in farmworker households in Salinas, California. Am J Ind Med, 45(6), 491-499.

Guilhermino, L., Barros, P., Silva, M., & Soares, A. M. (1998). Should the use of inhibition of cholinesterases as a specific biomarker for organophosphate and carbamate pesticides be questioned. Biomarkers, 3(2), 157-163.

Handal, A. J., Hund, L., Paez, M., Bear, S., Greenberg, C., Fenske, R. A., & Barr, D. B. (2015). Characterization of Pesticide Exposure in a Sample of Pregnant Women in Ecuador. *Arch Environ Contam Toxicol*. doi: 10.1007/s00244-015-0217-9

Janhong, K., Lohachit, C., Butraporn, P., & Pansuwan, P. (2005). Health promotion program for the safe use of pesticides in Thai farmers.

Jintana, S., Sming, K., Krongtong, Y., & Thanyachai, S. (2009). Cholinesterase activity, pesticide exposure and health impact in a population exposed to organophosphates. Int Arch Occup Environ Health, 82(7), 833-842.

Lekei, E. E., Ngowi, A. V., & London, L. (2014). Farmers' knowledge, practices and injuries associated with pesticide exposure in rural farming villages in Tanzania. *BMC Public Health, 14*, 389. doi: 10.1186/1471-2458-14-389

Lorenz, A. N., Prapamontol, T., Narksen, W., Srinual, N., Barr, D. B., & Riederer, A. M. (2012). Pilot study of pesticide knowledge, attitudes, and practices among pregnant women in northern Thailand. *International journal of environmental research and public health, 9*(9), 3365-3383.

London, L., De Grosbois, S., Wesseling, C., Kisting, S., Rother, H. A., & Mergler, D. (2002). Pesticide Usage and Health Consequences for Women in Developing Countries: Out of Sight Out of Mind? Int J Occup Environ Health, 8(1), 46-59.87

Mnif, W., Hassine, A. I. H., Bouaziz, A., Bartegi, A., Thomas, O., & Roig, B. (2011). Effect of endocrine disruptor pesticides: a review. *International Journal of Environmental Research and public health, 8*(6), 2265-2303.

Muñoz-Quezada, M. T., Lucero, B. A., Barr, D. B., Steenland, K., Levy, K., Ryan, P. B., . . . Rojas, E. (2013). Neurodevelopmental effects in children associated with exposure to organophosphate pesticides: a systematic review. *Neurotoxicology, 39*, 158-168.

Naksen, W., Prapamontol, T., Mangklabruks, A., Chantara, S., Thavornyutikarn, P., Srinual, N., . . . Barr, D. B. (2015). Associations of maternal organophosphate pesticide exposure and PON1 activity with birth outcomes in SAWASDEE birth cohort, Thailand. *Environ Res, 142*, 288-296. doi: 10.1016/j.envres.2015.06.035

Oesterlund, A. H., Thomsen, J. F., Sekimpi, D. K., Maziina, J., Racheal, A., & Jors, E. (2014). Pesticide knowledge, practice and attitude and how it affects the health of small-scale farmers in Uganda: a cross-sectional study. *Afr Health Sci, 14*(2), 420-433. doi: 10.4314/ahs.v14i2.19

Panuwet, P., Prapamontol, T., Chantara, S., Thavornyuthikarn, P., Montesano, M. A., Whitehead, R. D., & Barr, D. B. (2008). Concentrations of urinary pesticide metabolites in small-scale farmers in Chiang Mai Province, Thailand. *Sci Total Environ, 407*(1), 655-668.

Panuwet, P., Prapamontol, T., Chantara, S., & Barr, D. B. (2009). Urinary pesticide metabolites in school students from northern Thailand. Int J Hyg Environ Health, 212(3), 288-297.

Panuwet, P., Siriwong, W., Prapamontol, T., Ryan, P. B., Fiedler, N., Robson, M. G., & Barr, D. B. (2012). Agricultural pesticide management in Thailand: status and population health risk. *Environ Sci Policy, 17*, 72-81.

Prapamontol, T., Sutan, K., Laoyang, S., Hongsibsong, S., Lee, G., Yano, Y., . . . Panuwet, P. (2014). Cross validation of gas chromatography-flame photometric detection and gas chromatography–mass spectrometry methods for measuring dialkylphosphate metabolites of organophosphate pesticides in human urine. Int J Hyg Environ Health, 217(4), 554-566.

Quandt, S. A., Hernández-Valero, M. A., Grzywacz, J. G., Hovey, J. D., Gonzales, M., & Arcury, T. A. (2006). Workplace, household, and personal predictors of pesticide exposure for farmworkers. Environ Health Perspect, 943-952.

Quiros-Alcala, L., Alkon, A. D., Boyce, W. T., Lippert, S., Davis, N. V., Bradman, A., . . . Eskenazi, B. (2011). Maternal prenatal and child organophosphate pesticide exposures and children's autonomic function. *Neurotoxicology, 32*(5), 646-655. doi: 10.1016/j.neuro.2011.05.017

Reiss, R., Chang, E. T., Richardson, R. J., & Goodman, M. (2015). A review of epidemiologic studies of low-level exposures to organophosphorus insecticides in non-occupational populations. *Crit Rev Toxicol, 45*(7), 531-641. doi: 10.3109/10408444.2015.1043976

Sam, K. G., Andrade, H. H., Pradhan, L., Pradhan, A., Sones, S. J., Rao, P. G., & Sudhakar, C. (2008). Effectiveness of an educational program to promote pesticide safety among pesticide handlers of South India. *Int Arch Occup Environ Health, 81*(6), 787-795.

Sudakin, D. L., & Stone, D. L. (2011). Dialkyl phosphates as biomarkers of organophosphates: the current divide between epidemiology and clinical toxicology. Clin Toxicol, 49(9), 771-781.

Suratman, S., Edwards, J. W., & Babina, K. (2015). Organophosphate pesticides exposure among farmworkers: pathways and risk of adverse health effects. *Rev Environ Health, 30*(1), 65-79. doi: 10.1515/reveh-2014-0072

Thapinta, A., & Hudak, P. F. (2000). Pesticide use and residual occurrence in Thailand. *Environ Monit Assess, 60*(1), 103-114.

Winship, C., & Mare, R. D. (1984). Regression models with ordinal variables. Am Sociol Rev, 512-525.

Yang, M., Park, M. S., & Lee, H. S. (2006). Endocrine disrupting chemicals: human exposure and health risks. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev, 24*(2), 183-224. doi: 10.1080/10590500600936474

TABLES

Table 1: Demographic characteristics of the SAWASDEE Pilot Birth Cohort (n = 56)

	Mean	SD	Range
Age	26.3	4.7	18 - 35
Gestational Age at Enrollment	12.9	3.4	7 -19
Gestational Age at Birth[1]	38.1	1.2	35 - 41
Number of Measurement Occasions	8.2	1.8	5 -13
	n	%	
Marital Status			
Married	5	8.9	
Living as married	51	91.1	
Ethnicity			
Thai	11	19.6	
Thai Yai	34	60.7	
Chinese	2	3.6	
Palong	3	5.4	
Muser	4	7.1	
Larhu	2	3.6	
Language			
Northern Thai	15	26.8	
Thai Yai	33	58.9	
Other	8	14.3	
Education			
None	36	64.3	
Primary School (P1-6)	10	17.9	
Secondary School (M1-3)	2	3.6	
Upper Secondary School (M4-6), no diploma	7	12.5	
Attended some college	1	1.8	
Income[2]			
≤ 1,500 Baht	1	1.8	
1,501 - 3,000 Baht	9	16.1	
3,001 - 6,000 Baht	27	48.2	
6,001 - 9,000 Baht	10	17.9	
9,001 - 12,000 Baht	3	5.4	
> 12,000 Baht	3	5.4	
Not reported	3	5.4	
Parity			
Zero	21	37.5	
One	23	41.1	
Two	7	12.5	
Three	5	8.9	
Season of Enrollment			
Dry (November - February)	13	23.2	
Hot (March - June)	24	42.9	
Rainy (July - October)	19	33.9	

[1] 7 (12.5%) of the mothers had a child that born preterm (defined as less than 37 gestational weeks)

[2] 37 (66.1%) of the mothers live under the international poverty line of 6,000 baht per year

Table 2. Knowledge, Attitude, and Practice Scores

	n	Mean	SD	Range
Knowledge Score				
Knowledge Score, Total[1] (0 - 49)	56	39.2	3.7	29 - 44.5
Knowledge Score, Baseline	56	37.6	5.9	16 - 45
Knowledge Score, 24 Weeks	56	40.8	3.0	31 - 46
Attitude Scores				
Personal Susceptibility Score, Total[1] (0 - 4)	56	2.9	1.3	0 - 4
Personal Susceptibility Score, Baseline	56	2.6	1.6	0 - 4
Personal Susceptibility Score, 24 Weeks	56	3.3	1.4	0 - 4
Child Susceptibility Score, Total[1] (0 - 8)	56	6.1	1.6	2 - 8
Child Susceptibility Score, Baseline	56	6.0	2.1	2 - 8
Child Susceptibility Score, 24 Weeks	56	6.2	1.9	2 - 8
Responsibility Score, Total[1] (0 - 12)	56	9.1	1.7	5.5 - 12
Responsibility Score, Baseline	56	8.7	2.3	4 -12
Responsibility Score, 24 Weeks	56	9.6	1.8	5 - 12
Usefulness Score [2] (0 - 13)				
Usefulness Score, Baseline	31	5.3	2.5	1 - 11
Practice Scores				
Risky Behaviors at Work Score [3] (0 - 3)				
Risky Behaviors at Work Score, Baseline	54	0.9	0.7	0 - 2
Risky Behaviors at Work Score, 24 Weeks	40	0.5	0.7	0 - 2
Risky Behaviors at Work Score, Delivery	9	0.7	0.9	0 - 2
Risky Behaviors at Home Score (0 - 7)				
Risky Behaviors at Home Score, Baseline	56	2.3	1.4	0 - 7
Risky Behaviors at Home Score, 24 Weeks	56	2.3	1.2	0 - 5
Risky Behaviors at Home Score, Delivery	56	2.1	1.3	0 - 5

[1] Total score was calculated by using mean of baseline and 24 week scores

[2] Usefulness score was only calculated for mothers who have personally sprayed or applied pesticides

[3] Risky behaviors at work score was only calculated for mothers who worked at time of questionnaire

Table 3. Average diakylphosphate (DAP) metabolite levels by trimester (nmol/L)

	n	Mean	SD	Median	Range
Total					
DAP	56	372.8	394.5	208.9	42.4 - 1,759.4
DEAP	56	331.4	383.8	152.9	20.8 - 1,736.5
DMAP	56	41.5	66.6	24.0	16.6 - 414.6
First Trimester					
DAP	34	558.4	966.0	128.9	27.5 - 3,854.9
DEAP	34	521.9	963.0	93.3	9.8 - 3,823.4
DMAP	34	36.5	46.6	17.3	16.1 - 271.2
Second Trimester					
DAP	56	371.9	480.5	210.8	29.6 - 2,897
DEAP	56	329.0	468.5	152.6	12.3 - 2,889.0
DMAP	56	49.0	104.9	17.9	15.4 - 723.7
Third Trimester					
DAP	56	323.5	390.3	134.9	27.8 - 1,565.7
DEAP	56	288.2	369.3	107.7	10.6 - 1,548.5
DMAP	56	35.3	66.8	18.1	17.3 - 514.7

Table 4. Simple linear regression models between pesticide KAP scores and urinary $\log_{10}$ DAP levels

	First Trimester		Second Trimester		Third Trimester		Total	
	β	p-value	β	p-value	β	p-value	β	p-value
Knowledge Score								
Knowledge Score, Total[1]	-0.01558	0.72	-0.020	0.43	-0.03573	0.09*	-0.01786	0.31
Knowledge Score, Baseline	-0.00727	0.79	-0.01683	0.38	-0.02655	0.06*	-0.01535	0.20
Knowledge Score, 24 Weeks	-0.0119	0.77	-0.00504	0.79	-0.01725	0.47	-0.00283	0.89
Attitude Scores								
Personal Susceptibility Score, Total	-0.019	0.85	-0.025	0.62	-0.008	0.88	-0.010	0.83
Personal Susceptibility Score, Baseline	-0.030	0.69	-0.027	0.49	-0.046	0.29	-0.026	0.46
Personal Susceptibility Score, 24 Weeks	0.012	0.89	-0.005	0.92	0.048	0.34	0.019	0.64
Child Susceptibility Score, Total	0.057	0.43	0.008	0.85	-0.023	0.61	0.019	0.62
Child Susceptibility Score, Baseline	0.003	0.96	-0.023	0.45	-0.052	0.12	-0.011	0.70
Child Susceptibility Score, 24 Weeks	0.067	0.24	0.039	0.25	0.031	0.40	0.038	0.21
Responsibility Score, Total	0.001	0.98	-0.062	0.10*	-0.081	0.05**	-0.062	0.07*
Responsibility Score, Baseline	-0.014	0.79	-0.045	0.10*	-0.037	0.23	-0.033	0.19
Responsibility Score, 24 Weeks	0.021	0.73	-0.038	0.28	-0.087	0.03**	-0.058	0.07*
Usefulness Score								
Usefulness Score, Baseline	0.101	0.10*	0.068	0.06*	0.079	0.03**	0.065	0.04**
Practice Scores								
Risky Behaviors at Work Score[2]								
Risky Behaviors at Work Score, Baseline	-0.281	0.12	-0.0831	0.32	-0.070	0.45	-0.11171	0.15
Risky Behaviors at Work Score, 24 Weeks	-	-	-0.022	0.86	-0.073	0.57	-0.0809	0.47
Risky Behaviors at Work Score, Delivery	-	-	-	-	-0.065	0.82	-0.18929	0.27
Risky Behaviors at Home Score[2]								
Risky Behaviors at Home Score, Baseline	-0.131	0.19	-0.07624	0.11	-0.07706	0.14	-0.079	0.08*
Risky Behaviors at Home Score, 24 Weeks	-	-	-0.009	0.86	0.024	0.68	0.004	0.94
Risky Behaviors at Home Score, Delivery	-	-	-	-	-0.022	0.68	0.015	0.73

* Statistically significant at $\alpha = .10$; ** Statistically signficant at $\alpha = .05$

[1] Both knowledge scores adjusted for maternal income level

[2] All risky behavior scores adjusted for maternal income level and parity