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Prenatal Chlorpyrifos Exposure and Head Circumference in the SAWASDEE Maternal Birth Cohort

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By

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B.S.
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Abstract

Prenatal Chlorpyrifos Exposure and Head Circumference in the SAWASDEE Maternal Birth Cohort

By Emily McDonald

Background: Research suggests that prenatal exposure to Chlorpyrifos (CPF) is associated with adverse birth outcomes and may impair infant neurodevelopment. This thesis aimed to assess the relationship between average maternal urinary 3,5,6-trichloro-2-pyridinol (TCPy) concentrations, which are a specific metabolite of CPF, maternal paraoxonase 1 (PON1) phenotype, and infant head circumference at birth. This thesis supports ongoing research with the Study of Asian Women And their offSpring's Development and Environmental Exposures (SAWASDEE), which is a maternal birth cohort comprised of 322 pregnant female farmworkers in Northern Thailand.
Methods: Multiple linear regression models were used to assess for associations between average TCPy concentrations throughout pregnancy, PON1 phenotype, and head circumference. Covariates were identified a priori using literature review and directed acyclic graphs. Final model covariates were PON phenotype, smoking status, location, gestational age, maternal age, maternal height, maternal BMI at enrollment, income, and infant sex.
Results: On average, head circumference at birth decreased by 0.22 cm for every 100 ng/mL change in maternal urinary TCPy, adjusting for covariates ($CI_{95\%}$: -4.7, 0.03; p=0.08). PON1 did not modify the association between prenatal CPF exposure and head circumference.
Conclusions: These results suggest that prenatal CPF exposure is associated with head circumference at birth regardless of PON1 phenotype. This finding is of marginal significance given the limited sample size and power and warrants the need for additional research and appropriate intervention.

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I. Background

Chlorpyrifos

Chlorpyrifos (CPF) is a type of organophosphate (OP) insecticide produced by esterification between alcohols and phosphoric acid (Adeyinka et al, 2022). CPF is designed to be neurotoxic by irreversibly binding acetylcholinesterase in nervous tissues, preventing the breakdown of the neurotransmitter acetylcholine. Acute OP neurotoxicity is mediated by accumulation of acetylcholine in the peripheral and central cholinergic synapses which leads to overactivation of the nicotinic and muscarinic receptors, resulting in cholinergic hyperstimulation, and subsequent receptor down-regulation and neuronal death (Costa 2006). Early symptoms of acute OP poisoning include salivation, hypotension, seizures, coma, and respiratory failure (Blain 2011).

Although AChE inhibition is the primary mechanism of CPF toxicity, CPF and other OP insecticides exert more subtle toxic effects through other mechanisms below the threshold for AChE inhibition. Low-dose chronic exposures can lead to neuroinflammation, oxidative stress, and perturbations in neurotransmitter function. Some studies suggest that low-dose exposure to CPF increases risk for neurodegenerative disease including Alzheimer's disease and Parkinson's disease (Baldi et al. 2003; Richardson et al. 2014; Liu et al. 2019).

Low-dose CPF exposure has also been shown to impair neurodevelopment. Infants and children are considered high risk of the neurotoxic effects of low levels of CPF and other OPs. OP exposure early in life is associated with reduced cognitive ability and IQ, neurobehavioral problems, and problems with attention and working memory (Eskenazi et al. 2007; Bouchard et al. 2011; Rauch et al. 2012). A growing number of studies have focused on exposure to OPs

among pregnant women and human fetuses (Bradman et al. 2003, Perera et al. 2003; Whyatt et al. 2003; Eskenazi et al. 2004; Wolff et al. 2007; Barr et al. 2007; Rauh et al. 2012). Prenatal exposure to organophosphates is particularly harmful because OP insecticides can transfer to the fetus via the placenta. Fetuses are more susceptible to the deleterious effects of OP exposure than adults due to the underdeveloped and rapidly growing brain (Rice 2000). Moreover, detoxification mechanisms do not develop fully for years, so fetuses and infants have few defenses against neurotoxic insults. Animal models suggest CPF causes developmental neurotoxicity by disrupting neuronal differentiation, proliferation, and migration, synaptogenesis and axonogenesis, and synaptic function at doses below the threshold for significant AChE inhibition (Aldridge et al, 2005). Growing evidence from epidemiological studies suggest prenatal exposure to OP during critical windows of susceptibility has lasting adverse effects on growth and cognition (Sapbamrer R, Hongsibsong S, 2019; Nuseir et al. 2022). Although the underlying pathophysiology remains uncertain, recent studies point to changes in placental gene-networks and perturbations in signaling pathways involved in inflammation, oxidative stress, and neural development (Liang et al. 2022; Li et al. 2023)

Several studies have investigated the relationships between prenatal exposure to organophosphate and adverse birth outcomes such as birth weight, length, and head circumference because these have been found to be important predictors of growth and subsequent cognitive ability (Harley et al. 2016. Khoshhali et al. 2020). Prenatal OP exposure has been shown to be associated with shorter gestational age, lower birth weight (Rauch et al. 2012; Eskenazi et al. 2004). Few studies have examined head circumference as the outcome of interest. In utero CPF exposure has been shown to be negatively associated with reduced fetal head circumference (Wolff et al. 2007). However, this study did not find any associations with

head circumference at birth. Other studies have found no association between prenatal CPF exposure and head circumference at birth (Eskenazi et al. 2004; Marks et al. 2010; Engel et al. 2011, Horton et al. 2011). Findings have been inconsistent due to differences in study design, exposure assessment methods, and exposure scenarios. The lack of consistent findings exploring relationships between head circumference and prenatal exposure to Chlorpyrifos suggests more research is needed.

3,5,6-trichloro-2-pyridinol (TCPy)

Absorption of CPF and other OP insecticides occurs primarily through the respiratory system, the gastrointestinal tract, or the skin. Upon absorption, they are distributed throughout the body including the brain, muscles, liver, and kidneys. Cytochrome p450 enzymes quickly metabolize CPF to the activated and neurotoxic oxon form, which exhibits inhibitory cholinesterase activity. CPF-oxon exerts toxic effects primarily by irreversibly binding acetylcholinesterase (AChE), resulting in accumulation of acetylcholine and overstimulation of the nervous system. However, CPF and CPF metabolites exert other toxic effects even in low-dose exposures below the threshold for AChE inhibition primarily through pathways involved in inflammation and oxidative stress. Unbound CPF may be hydrolyzed by paraoxinase 1 (PON1) or it may hydrolyze spontaneously to form chlorpyrifos specific 3,5,6-trichloro-2-pyridinol (TCPy) and nonspecific dialkyl phosphate (DAP) metabolites (Figure 1) (Timchalk et al. 2002; Androutsopoulos et al. 2011). TCPy may then undergo phase II conjugation reactions catalyzed by UDP-glucuronosyltransferases (UGTs) and sulfotransferases (SULTs). UGTs and SULTs transfer glucuronic acid and a sulfate group, respectively, to the hydroxyl group of TCPy (Cost et al. 2008). Phase II conjugation makes the TCPy conjugates more water soluble, which facilitates elimination. Unconjugated TCPy and DAP metabolites or their sulfonated or glucuronidated

conjugates are then excreted in the urine (Muñoz-Quezada et al, 2013). OP metabolism is similar for other forms of OP, and most OP metabolites including TCPy have short biological half-lives. Most of the metabolites are eliminated through the urine within a few days (Lockridge et al, 2019). Urinary TCPy is frequently used as a biomarker of exposure to CPF due to its specificity to CPF or its dimethyl analogue, relative stability in urine, and ease of sample collection. Non-specific DAP metabolites of CPF include diethylphosphate (DEP) and diethylthiophoshpate (DETP).

PON1

Paraoxonase 1 (PON1) is frequently used as a biomarker of susceptibility to OP toxicity because it is one of the major metabolizing enzymes involved in OP metabolism (Costa et al, 2003). PON1 is thought to play a critical role in the detoxification of CPF and other OP pesticides by hydrolyzing the activated oxon CPF metabolite to form TCPy and other DAP metabolites (Figure 1). The serum level of PON1 is highly variable due partly to the single-nucleotide polymorphisms of the *PON1* gene. The polymorphism at codon 192 (Q192R) is caused by a substitution of glutamine (Q) with arginine (R), which affects the serum activity of the PON1 enzyme towards different OP pesticides (Harel et al. 2004). The substitution of glutamine with arginine alters its substrate affinity causing PON1 to have increased paraoxonase activity and decreased arylesterase activity (Taler-Verčič et al. 2020). This affects the enzymes' ability to detoxify CPF and other OP insecticides. Measuring the ratios of paraoxonase to arylesterase affinities in the serum reveals a polymorphic distribution of paraoxonase serum activity indicating three defined phenotypes of low, intermediate, and high paraoxonase activity, which correspond to the QQ, QR, and RR genotypes (Huen et al. 2009; Mueller et al, 1983).

Previous research suggests that individuals with the R allele of the PON1 gene have greater protection against CPF oxon (Li et al. 2000). Several studies have investigated the role of maternal PON1 phenotype on CPF and birth outcomes. However, the results have been mixed. A study by Engel et al. (2007) found that maternal PON1 genotype modified the association between CPF exposure and birth weight. Among mothers with the PON1-192QQ genotype, there was a significant decrease in birth weight associated with higher CPF exposure. However, no significant association between CPF and birth weight was observed among mothers with the PON1 QR or RR phenotypes (Engel et al. 2007). Another study by Rauch et al found that birthweight was inversely associated with prenatal OP exposure with the strongest effect among infants born to mothers with the PON1-192QR phenotype and no significant association between CPF and birthweight among mothers with the RR or QQ phenotypes (Rauch et al. 2012).

Fewer studies have evaluated this effect by PON1 on head circumference as the birth outcome. A study by Whyatt et al. (2004) found CPF exposure was inversely related to head circumference regardless of PON1 phenotype. Another study found an inverse relationship between head circumference and CPF exposure that was stronger among infants born to mothers with PON1-192QQ phenotype and lower PON1 activity (Berkowitz et al. 2004). More research is needed to elucidate the role of PON1 phenotype on CPF exposure and head circumference.

Thailand Organophosphate Use

Despite the well-documented neurotoxic effects, CPF and other OP insecticides continue to be some of the most abundantly used pesticides worldwide. OP insecticides are used to reduce the spread of zoonotic diseases. OP insecticides are also frequently used in medicine, agriculture, and household products worldwide. As an agricultural country and one of the top exporters of

commodity crops in the world, Thailand depends on OP and other pesticides to increase crop yields and meet global demand (Panuwet et al, 2012). Due to rapidly expanding agricultural activities and poor regulation, pesticide use in Thailand has increased dramatically over the past few decades with the heaviest distribution in rural areas in central, north, northeastern parts of Thailand (Laohaudomchok et al. 2020). An estimated 38% of the Thai population work in agriculture with women making up 60% of agricultural workers (Kongtip et al 2018). Reports from the Ministry of Public Health suggest cases of pesticide poisoning have steadily decreased since 2017. However, these reports likely underestimate the true number of cases because they do not include cases from informal farming activities (Laohaudomchok et al 2020). Moreover, many farmers apply excessive amounts of pesticides and do not use appropriate personal protective equipment (Rivera et al, 2016; Punkhun et al. 2020). Pregnant farmworkers are among the most vulnerable groups to the harmful effects of OP. In Thailand, they often work throughout pregnancy leading to potentially high exposure scenarios due to frequent and overuse of OP, proximity to application sites, and the unique vulnerability of the developing fetus.

SAWASDEE Birth Cohort

The word “sawasdee” is a common greeting or farewell in Thailand. The Study of Asian Women and their Offspring’s Development and Environmental Exposures (SAWASDEE) was designed to address the knowledge gaps that have prevented effective implementation of policies in Thailand. The SAWASDEE study is a longitudinal birth cohort study that follows farmworker women in the Chiang Mai Province of Thailand and their infants. Women were enrolled during their first trimester of pregnancy to evaluate their exposure to pyrethroid and organophosphate

insecticides. Infant growth and neurodevelopment were assessed multiple times using multiple indices until 3 years of age.

This thesis supports ongoing research with the SAWASDEE maternal birth cohort. Although growing evidence suggests CPF exposure leads to adverse birth outcomes and has long-term effects on neurodevelopment, research findings exploring the relationship between CPF exposure and head circumference remain unclear and inconsistent. Research aims are to elucidate the relationship between head circumference at birth and prenatal CPF exposure among infants born to female Thai farmworkers with varying biological susceptibility to OP toxicity.

Aims and Hypotheses

The first aim is to investigate associations between average prenatal exposure to CPF throughout pregnancy and infant head circumference at birth. The hypothesis is that prenatal CPF exposure as indicated by mean maternal urinary TCPy concentration throughout pregnancy will be negatively associated with infant head circumference at birth. Due to the high susceptibility and vulnerability of the developing fetal brain, CPF exposure during the prenatal period may disrupt cranium development resulting in reduced head circumference.

The second aim is to explore if biological susceptibility indicated by PON1 phenotype modifies the association between prenatal CPF exposure and head circumference. If PON1 phenotype modifies the association between head circumference and mean urinary TCPy concentration, then this relationship will differ depending on PON phenotype. Because PON1 activity is critical in detoxification of OP insecticides and PON1 phenotype is a biomarker of susceptibility to OP toxicity, it follows that the most pronounced effect may be observed among women with low PON1 activity compared to those with intermediate and high metabolic activity.

II. Method and Materials

Participants and Recruitment

The cohort for the SAWASDEE study is comprised of women living in the Chiang Mai Province in northern Thailand who either work in agriculture or lived on a farm. This study focused on two agricultural districts, Fang and Chom Thong, outside Chiang Mai City. Recruitment occurred between July 2017 and June 2019. Eligibility criteria for participation in the study were the following: (1) spoke Thai at home, (2) overall good health, (3) worked in agriculture or lived within 50 m of a working farm, (4) lived in Fang or Chom Thong for at least 6 months and planned to stay in their district for at least 3 years, (5) had a ID card for clinic access, (6) did not use illegal drugs or drink more than 2 drinks daily, (7) presented with a gestational age of at least 16 weeks, and (8) were between 18 and 35 years old. 1289 women were assessed for eligibility using a screening questionnaire. Of the 1289 women who completed the recruitment questionnaire, 394 women were enrolled. Of the 394 participants, 334 women completed data collection to term with 225 participants residing in Chom Thong and 128 from Fang. Most women who did not complete data collection throughout their pregnancy were excluded because of pregnancy complications.

Questionnaires and Medical Record Extraction

Several questionnaires were administered in Thai to gather data on pregnant women. These questionnaires included an intake questionnaire to collect baseline data and extensive exposure questionnaires administered during each trimester. The exposure questionnaires covered demographics, medical histories, maternal nutrition, personal habits, household

characteristics, work-related tasks, and pesticide use. Overall, the questionnaires provided important information on the population's characteristics including education, medications, medical conditions, and supplements taken during pregnancy.

Medical records were used to track various indicators related to pregnancy and childbirth. These include details about the mother's past obstetric history, prenatal visit history, height and weight, and lifestyle habits such as smoking, drinking, and drug use. These data were collected at each visit to track maternal nutritional status and stressors that could affect the pregnancy. Additionally, birth records provided information regarding childbirth including the infant's sex, weight, length, head circumference, delivery method, and details on any maternal or neonatal complications.

Biological Samples and Exposure Assessment

Urine, blood, and serum were collected during each trimester. Blood was used for analyzing and phenotyping PON1 to measure maternal susceptibility to OP insecticides. Urine was the primary matrix used for exposure assessment. Composite maternal urine samples were analyzed using previously established analytical methods (Olsson et al, 2004). Urine specific gravity and creatinine concentrations were measured for urine matrix adjustments.

PON1 Enzyme Activity and Phenotyping

To measure PON1 activity, activity levels of paraoxonase (PONase) and arylesterase (AREase) were measured in plasma samples taken from maternal venous blood using Huen's method (Huen et al. 2009). PON1 phenotype was determined using Eckerson's method (Eckerson et al. 1983), which involved calculating the ratio of salt stimulated PONase activity to

AREase activity in maternal plasma samples. The resulting cumulative distribution plot (Figure 2) revealed clear cut points that separated the samples into three PON1 phenotypes of low, middle, and high activity, which correspond to QQ, QR, and RR genotypes, respectively, at amino acid 192 of the PON1 gene.

Statistical Analysis

Covariate Analysis

For all models, covariates were identified a priori from literature review and using directed acyclic graphs. Collinearity assessments and regression diagnostics were conducted to assess any major violations of regression model assumptions. Confounding assessment was used to assess for evidence of confounding with each variable individually. Variables that changed the estimate by more than 10 percent suggest evidence of confounding and were retained in the model. Final covariates were PON phenotype, smoking status, location, gestational age, maternal age, maternal height, maternal BMI at enrollment, income, and infant sex. A linear regression model with covariates was used to assess how much of the variance in head circumference was explained by the covariates. A likelihood ratio test was used to assess the goodness of fit comparing models with and without TCPy.

Aims

For Aim 1, multiple linear regression models were used with the means of log-transformed urinary concentrations of TCPy throughout pregnancy as the exposure and head circumference at birth as the outcome. The mean urinary concentrations of TCPy were calculated

from all maternal samples for each participant. Urine log creatinine and specific gravity were model adjusted individually. Confidence intervals indicate precision of estimates.

For Aim 2, similar models were run stratifying by PON1 phenotype. First, pairwise comparisons were done assessing for significant differences between mean head circumference among each phenotype (Figure 3). Likelihood ratio tests were used to assess if the association between head circumference and TCPY differed depending on phenotype. The hypothesis is that the association between TCPy and head circumference will differ according to maternal PON1 phenotype with the smallest effect among mothers with high PON1 activity compared to mothers with low PON1 activity. If high PON1 activity mitigates the toxicity of CPF, then a smaller effect will be observed among high metabolizers.

III. Results

Descriptive Characteristics

Table 1 presents the descriptive characteristics of the SAWASDEE cohort in Thailand from 2017-2019 (Baumert et al. 2022). The cohort consisted of 322 mothers with 216 from Chom Thong and 106 from Fang. The mean age of mothers at enrollment was 25.0 years, with a range of 18 to 39 years. The mean body mass index (BMI) of mothers at the first visit was 22.9 kg/m2. Most mothers were from Thailand (86%) and able to read and write in Thai (80.4% and 81.4%, respectively). Most mothers had formal education between no education and some high school (87%). The mean monthly income was 10615.1 Thai baht, and the mean number of people living in households was 5.2. Most mothers were living as married (83.4%). 15.8% of mothers smoked at some point during pregnancy. 80.4% of mothers worked in agriculture during pregnancy. The sex of infants was evenly split between males and females. The mean gestational

age at birth was 38.5 weeks, and the mean birth weight was 3.0 kg. Most deliveries were vaginal (80%). Variables that were excluded from this table due to small cell counts were depression level, anxiety, alcohol use, and drug use.

Linear Regression Analyses

Covariate Analysis

Table 2 provides the results of the regression models of covariates adjusted for in analyses of associations between TCPy and head circumference. The covariates explained 26% of the variance in head circumference (adjusted $R^2 = 0.26$, p-value < 0.001). Income, sex, location, and gestational age were all significantly associated with head circumference.

Aim 1

Four regression models were used to assess the relationship between TCPy and head circumference: (1) a urine matrix model adjusted for the log of mean creatinine, (2) a urine matrix model adjusted for mean specific gravity, and (3) a regression model without matrix model adjustments (4) a regression model with mean TCPy as a predictor adjusted for log of mean urinary creatinine (Table 3). The first three models in Table 3 show the change in head circumference in centimeters for each log unit increase in average maternal urinary TCPy throughout pregnancy. All models were adjusted for PON phenotype, smoking, location, gestational age, maternal age, income, BMI at enrollment, height, and infant sex.

For the first two models, which adjusted for urinary log of mean creatine and mean specific gravity, the coefficients for XlTCPY (log unit increase in mean maternal urinary TCPy) were -0.22 and -0.22, respectively. Urinary creatinine had a greater confounding effect compared to specific gravity. The log mean creatinine adjusted models also had better model fits. The

model adjusting for log creatinine showed a negative association between head circumference and exposure to CPF, as indicated by the average log TCPy excreted in urine throughout the pregnancy, after adjusting for covariates. On average, head circumference at birth decreased by 0.22 cm for every 100 ng/mL change in maternal urinary TCPy, adjusting for covariates [B=-0.22; 95% CI: -4.7, 0.03; p=0.08]. The likelihood ratio test comparing the likelihood of model fit with and without XlTCPy suggests the difference in the goodness of fits after adding XlTCPy is significant at an alpha of 0.10 [LRT p = 0.08].

Notably, the fourth model suggests there is a significant association between average maternal urinary TCPy and head circumference. On average, head circumference at birth decreased by -0.028 cm for every ng/mL change in maternal urinary TCPy, adjusting for covariates [B=-0.028; 95% CI: -0.054, -0.0260; p = 0.03].

Aim 2

For aim 2, mean head circumference by PON phenotype is shown in Table 4 and Figure 3. Table 4 shows the mean head circumference by PON1 phenotype, with values for type 1, type 2, and type 3. The mean head circumference is lower for type 1 (32.72 cm) compared to type 2 (32.93 cm) and type 3 (32.92 cm). However, mean head circumference did not differ significantly by PON1 phenotype.

PON1 did not modify the effect on the association between head circumference and prenatal CPF exposure. Table 5 shows the results of the test for effect measure modification of PON1 on the association between mean head circumference and a 10-fold increase in average maternal urinary TCPy concentrations. Contrasts between type1 vs type2, type1 vs type3, and type2 vs type3 are shown. The B coefficients represent the differences between the means of head circumference and average log TCPy levels for each contrast. P values are for TCPy x

PON1 interactions. The family error rate was adjusted using Bonferroni corrections. No p values were statistically significant.

IV. Discussion

Interpretations

The findings presented in this thesis suggest a small but meaningful association between CPF exposure, as indicated by maternal urinary TCPy throughout pregnancy, and head circumference. For every 10-fold increase in maternal urinary TCPy, head circumference decreased by 0.22 cm. Although small, these changes can have long-term consequences on cognitive ability, behavior, and development. This finding is of proximal significance given the relatively small cohort size. Replication in a higher-powered study with a greater sample size will likely yield statistically significant associations between prenatal CPF exposure and head circumference.

The relationship between CPF exposure and head circumference did not differ dependently on biological susceptibility of mother as indicated by her PON1 phenotype. High metabolism of CPF through high maternal PON1 activity did not provide a protective effect against reduced head circumference nor did low metabolic PON1 activity confer a stronger effect with CPF exposure. This might be due to the limited power and sample size in this study.

Despite differences in population demographics and exposure assessment methods, comparable results were observed in several maternal birth cohorts with similar study designs. Table 6 summarizes research findings for four maternal birth cohorts in New York, Ohio, California, and Thailand. Four studies found inverse relationships between CPF exposure and head circumference at birth (Engel et al. 2007; Lanphear et al. 2011; Harley et al. 2011; Naksen

et al. 2015) or fetal head circumference (Wolff et al 2007). However, others found no association between CPF exposure and head circumference (Eskenazi et al 2004; Rauh et al 2006). Moreover, most of the studies that assessed head circumference used a sum of DAP metabolites as the biomarker of exposure for OP whereas this study used TCPy, which is specific for CPF. Although CPF is the primary OP used in Northern Thailand, other OP and PYR are used abundantly. Perhaps the combined effect of multiple OP exposures results in a larger effect size that is easier to detect in relatively small sample sizes compared to the individual effect of CPF. Meta analysis and other high-powered studies may be needed to elucidate the relationship between prenatal CPF and head circumference.

Strengths and Limitations

The SAWASDEE study was conducted using highly sensitive exposure assessment methods to obtain robust outcome data. While many similar cohort studies are limited to only one urine sample per participant, this study was able to achieve higher power by collecting multiple samples throughout pregnancy. Additionally, TCPy is specific to CPF, which reduced the risk of misclassification and allowed for investigation into the effects of CPF apart from other OP.

Retention rate for the SAWASDEE study was also considerably high, which speaks to the community-centered collaboration between the Fang and Chom Thong communities and the research team. This study also contributes to exposure assessments conducted in low- and middle-income countries, which are too often neglected.

This study is not without limitations. Live birth bias is one limitation because only live births were included in this study. The findings presented here may underestimate the true

association between head circumference and CPF exposure because only infants who survived to birth were included, which may have excluded infants who died shortly before or after birth in part due to complications linked to CPF exposure. Another limitation is that TCPy can be derived from preformed metabolites in the environment, which means exposure misclassification is possible. However, bias was minimized because the cohort was an occupational cohort with known activities of application.

V. Conclusion

Summary

This thesis shows that prenatal CPF exposure is negatively associated with head circumference regardless of the mother's biological susceptibility indicated by PON1 phenotype. This contributes to the growing body of research that suggests exposures to these chemicals early in life, particularly during critical windows of susceptibility in the womb, can have lasting effects on developing humans. Reduced head circumference is often an indicator of fetal growth restriction, which increases the risk for neurological and cognitive impairments throughout childhood. The findings in this thesis support the SAWASDEE study's larger aims to evaluate the effects of PYR and OP insecticide exposures and neurodevelopmental outcomes from early infancy to early childhood. Indeed, the SAWASDEE study and other maternal birth cohorts are revealing compelling insights into the role of prenatal pesticide exposure and impaired growth and cognitive, motor, and behavioral development throughout childhood (Kim et al. 2020; Liu et al. 2021; Pilkington et al. 2021).

Future Directions

More research is needed to elucidate the relationship between prenatal CPF exposure, birth outcomes, and PON1. While growing evidence suggests prenatal CPF exposure is associated with reduced growth and developmental delays in childhood, considerably less research has explored head circumference and fetal growth restriction as mediating variables in the causal path between CPF exposure and these deficits. Moreover, the underlying pathophysiological mechanisms are not clear and warrant further investigation. This thesis examined mean CPF exposure throughout pregnancy, but additional research is needed to assess if differences in CPF exposure during each trimester modify the association. Indeed, the developing fetal nervous system is highly vulnerable during windows of susceptibility where even minute levels of neurotoxic substances can dramatically alter the inherently dynamic processes in neurogenesis. Additionally, because fetal growth restriction has been linked to numerous health problems, future research may explore associations between prenatal CPF exposure, fetal growth restriction, and physical and mental health disorders later in life.

This thesis was also limited to examining CPF exposure specifically, but vulnerable populations and those at risk for adverse birth outcomes are often exposed to a mixture of pesticides and other pollutants. More analysis is needed to examine interactions between multiple pollutants and their independent and combined effects on birth outcomes. Furthermore, it is imperative that analyses disaggregate race, socioeconomic status, and other social factors to examine exposure and outcome disparities that may result from systemic oppression. SAWASDEE provides a good model for community engagement.

As genomics and other ‘omics-based approaches continue to expand, future research may leverage large-scale analysis to investigate the role of PON1 polymorphisms in CPF toxicity and

subsequent health problems. PON1 is one of many enzymes involved in the detoxification mechanisms of CPF and other organophosphates. New research may explore how variations in gene expressions and the serum activities of other detoxifying enzymes such as paraoxonase 2, carboxylesterases, aryl esterases, and glutathione S -transferases affect birth outcomes and subsequent neurodevelopment. The robust biological data available from the SAWASDEE cohort provides numerous opportunities for integration of traditional environmental epidemiological methods with high through-put computational analysis to glean new insights into sensitive the effects of OP exposure on gene networks, the transcriptome, and metabolome at critical timepoints throughout pregnancy. Indeed, a recent study by Li et al. (2023) found significant associations between maternal urinary DEP concentrations and two placental gene modules. Another study by Liang et al (2022) used SAWASDEE biosamples and metabolomics to reveal disturbances in metabolites and pathways in the first trimester associated with CPF exposure (Liang et al. 2022). These recent studies are providing new insights into the underlying pathways and mechanisms involved in CPF toxicity and adverse birth outcomes.

While many questions remain unanswered, CPF exposure poses a major threat to health, particularly for agriculture workers, infants, and children in low-income settings.
Thailand is among many agricultural countries throughout the world that relies on pesticides including CPF and other OP insecticides with inadequate regulation to meet the global demand in agriculture production. Nevertheless, the growing evidence showing the deleterious effects of OP insecticides calls for policy reform and more stringent regulations of their use and for new innovations to reduce the need for them.

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VII. Tables

Table 1: SAWASDEE Cohort Characteristics

Descriptive characteristics of the SAWASDEE cohort, Thailand, 2017-2019				
Variable		**SAWASDEE (N=322)**	**Chom Thong (n=216)**	**Fang (n=106)**
Age of mother at enrollment (years), mean (SD) Min - Max		25.0 (5.3) 18 - 39	25.5 (5.3) 18 - 39	23.9 (5.0) 18 - 38
Body mass index (BMI) of mother at first visit (kg/m^2), mean (SD)		22.9 (5.3)	22.7 (4.9)	23.3 (6.2)
Maternal Height (cm), mean (SD)		153.8 (6.0)	154.5 (6.2)	152.2 (5.2)
Maternal birth country	Thailand, n (%)	272 (86)	210 (97)	41 (39)
	Myanmar, n (%)	45 (14)	4 (2)	62 (61)
Maternal education n (%)				
	None	58 (18.4)	12 (5.6)	46 (43.1)
	Primary 1-6	47 (14.4)	19 (8.9)	28 (26.1)
	Junior high/high school	106 (32.4)	86 (39.6)	20 (18.3)
	High school/did not graduate	70 (21.4)	63 (29.3)	7 (6.2)
	Diploma/technical school equivalent	28 (8.4)	24 (11.4)	4 (3.8)
	Attended college but did not graduate	1 (0.4)	1 (0.6)	1 (0.9)
	College graduate or more	12 (3.4)	11 (5.5)	0
Mother able to read Thai, n (%)				
	Yes	260 (80.4)	206 (95.1)	54 (50.9)
	No	62 (19.4)	10 (4.9)	52 (49.1)
Mother able to write in Thai, n (%)				
	Yes	262 (81.4)	205 (94.3)	57 (53.8)
	No	60 (18.4)	11 (5.7)	49 (46.1)
Income/month (Thai baht), mean (SD)		10615.1 (8336.9)	11572.4 (9499.0)	8709.6 (4818.4)

Number of people living in households, mean (SD)		5.2 (2.5)	4.9 (2.2)	5.7 (3.0)
Marital status, n (%)				
	Legally married	47 (14.4)	46 (21.9)	1 (0.9)
	Living as married	268 (83.4)	166 (76.6)	102 (96.2)
	Divorced	2 (0.4)	2 (0.2)	0
	Separated	3 (0.4)	0	3 (2.8)
	Widowed	2 (0.4)	2 (0.2)	0
Mother smoking any time during pregnancy, n (%)				
	Yes	51 (15.8)	47 (21.8)	4 (3.8)
	No	258 (77.0)	162 (75.0)	96 (90.6))
Mother working in agriculture during pregnancy, n (%)				
	Yes	258 (80.4)	182 (84.3)	76 (72.4)
	No	63 (19.6)	34 (15.7)	29 (27.6)
Sex of infant, n (%)	Male	159 (49.5)	105 (48.8)	54 (50.4)
	Female	162 (50.5)	110 (51.2)	52 (49.5)
Gestational age at birth (weeks), mean (SD)		38.5 (1.2)	38.5 (1.1)	38.4 (1.2)
Infant birth weight (kg), mean (SD)		3.0 (0.4)	3.0 (0.4)	2.9 (0.4)
Infant head circumference (cm), mean (SD)		32.8 (1.5)	32.6 (1.5)	33.3 (1.6)
Infant length (cm), mean (SD)		48.4 (2.6)	47.6 (2.4)	50.0 (2.2)
Delivery Type, n (%)	Vaginal	256 (80)	170 (79)	86 (81)
	Cesarean section	62 (19)	43 (20)	19 (18)

*The following covariates were not included because of small cell counts: depression level, anxiety, drug use, and alcohol use
This table was adapted with permission from Baumert et al. 2022

Table 2: Covariate Analysis

N = 268 Adj R^2=0.26 F = 9.1 p<0.001 Covariate	***B***	**SE**	***p* - value**
Intercept	13.40	35.93	0.71
lxcreat[a]	0.38	0.38	0.31
xsg[b]	-4.50	36.75	0.90
PON1 Type 2[c]	0.17	0.28	0.56
PON1 Type 3[c]	0.18	0.28	0.51
bmi at first visit	0.03	0.02	0.06
Height	0.02	0.01	0.24
Smoker[d]	0.05	0.22	0.81
location (Fang)*	1.02	0.19	<0.001
maternal age	0.02	0.02	0.26
Income*	2.2E-05	1.0E-05	0.03
Infant sex (female)*	-0.73	0.16	<0.001
Gestational age*	0.47	0.08	<0.001

[a] log means urinary creatinine
[b] mean urine specific gravity
[c] PON1 phenotype low metabolizer (Type 1) was the reference group
Type 1 = low metabolizer QQ
Type 2 = medium metabolizer QR
Type 3 = high metabolizer RR
*Infant sex, gestational age, income, and location were significantly associated with head circumference.

Table 3: Change in Head Circumference (cm) for each log unit increase in average maternal urinary TCPy throughout pregnancy

Urine matrix model adjusted for log of mean creatinine (xlcreat)			
Coefficients	***B***	**SE**	***p* value**
XlTCPY	-0.22	0.13	0.08•
lxcreat	0.55	0.22	0.01
Urine matrix model adjusted for mean specific gravity (xsg)			
Coefficients	***B***	**SE**	***p* value**
XlTCPY	-0.22	0.13	0.1
xsg	48.68	22.44	0.03*
Regression model without matrix model adjustments			
Coefficients	***B***	**SE**	***p* value**
XlTCPY	-0.06	0.11	0.58
Regression model with mean TCPy adjusted for log of mean creatinine (xlcreat)			
Coefficients	***B***	**SE**	***p* value**
XTCPY	-0.028	1.3 E -2	0.03*
lxcreat	0.48	0.2	0.02*

* $p < 0.05$
• $p < 0.10$
All models were adjusted for PON phenotype, smoking, location, gestational age, age, income, BMI at enrollment, height, and infant sex

Table 4: Mean head circumference (cm) by PON1 phenotype

Mean head circumference (cm) by PON1 phenotype			
PON1 phenotype	***B***	**SE**	***p* value**
Type1 – low metabolizer QQ phenotype	32.72	0.28	<0.001
type2 – medium metabolizer QR phenotype	32.93	0.15	<0.001
type3 – high metabolizer RR phenotype	32.92	0.14	<0.001

Table 5: Assessment of PON1 Effect Measure Modification on the Association between Head Circumference and TCPy

PON1 Phenotype Contrasts	**B**	**SE**	**Adj *p* value**
type1 vs type2	-0.21	0.29	0.75
type1 vs type3	-0.2	0.28	0.77
type2 vs type3	0.01	0.17	0.99

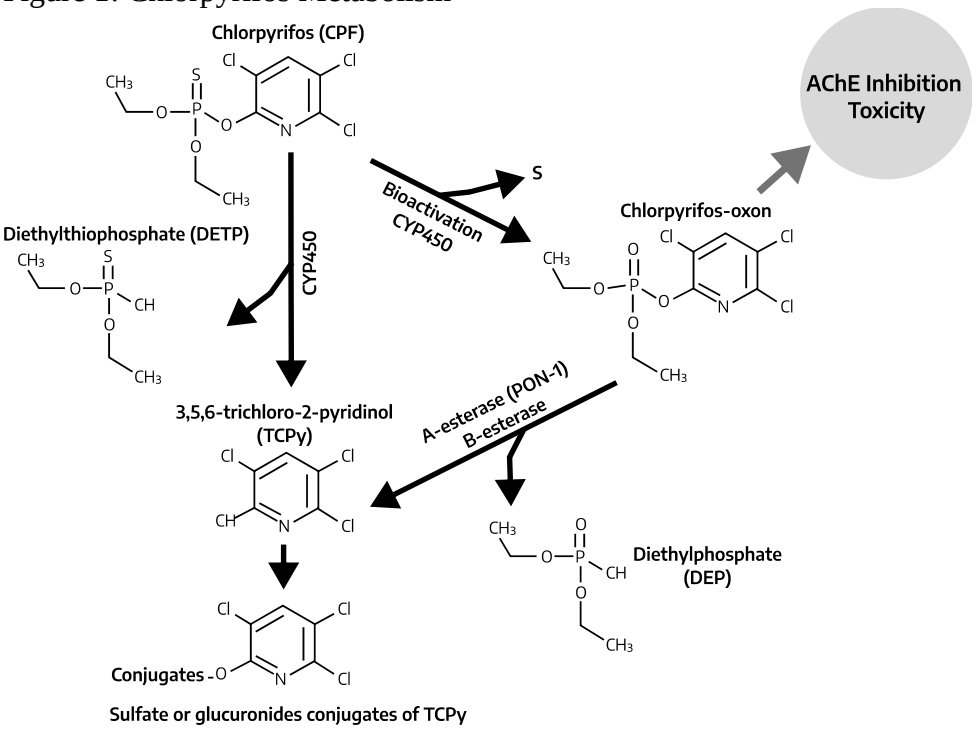
Table 6: Maternal Birth Cohort Study Comparison

Comparison of Maternal Birth Cohort Studies Assessing Prenatal CPF Exposure and Birth Outcomes				
Author, Year	**Population**	**Biomarkers**	**Birth Outcomes**	**Findings**
Columbia Center for Children's Environmental Health (CCCEH)				
Whyatt et al. 2004	low-income African American and Dominican; NYC	DAP, TCPy	birth weight and birth length	inverse association with birth length and birth weight
Perera et al 2005		Chord CPF	birth weight and birth length	inverse association with birth length and birth weight
Rauh et al. 2006		DAP, TCPY, PON1	birth weight, head circumference, gestational age	no associations with birth outcomes among general cohort; high exposure subset negatively associated with PON activity
Wolff et al. 2007		TCPy, PON1	fetal head circumference, fetal weight, femur length, abdominal circumference, birth weight, birth length, head circumference, gestational age	inverse association with fetal head circumference and increased odds of SGA; PON1 EMM - negative association between CPF metabolites and fetal head circumference among African American Subgroup of mothers with Q192R (type 2) genotype
Engel et al. 2007		DAP	birth weight, birth length, and head circumference	inverse association with head circumference
Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS)				
Eskenazi et al. 2004	low-income Mexican women in California	DAP	birth length, head circumference, gestational age	inverse relationship gestational age
Harley et al. 2011		fetal PON1, DAP	birth weight, head circumference, gestational age	inverse associations between fetal PON1 and gestational age and head circumference. EMM by DAP among those with susceptible genotype
Huen et al. 2018		DAP	birth weight and gestational age	inverse association with birth weight and gestational age
Health Outcomes and Measures of the Environment (HOME)				

Lanphear et al. 2011	Diverse population in Ohio, Indiana, and Kentucky	DMP and DEP	head circumference, birth weight, birth length, gestational age, Apgar scores	inverse association with head circumference
Rauch et al. 2012		∑DAP, infant PON1 phenotype	gestational age, birth weight	inverse association with gestational age; inverse association with birth weight; stronger effect among PON1-*192QR* phenotype
Study of Asian Women and their Offspring's Development and Environmental Exposures (SAWASDEE) Pilot				
Naksen et al. 2015	Fang Thailand	PON1 DEAP and DAP	head circumference, birth weight, birth length, gestational age, Apgar scores	inverse association with head circumference and birth weight

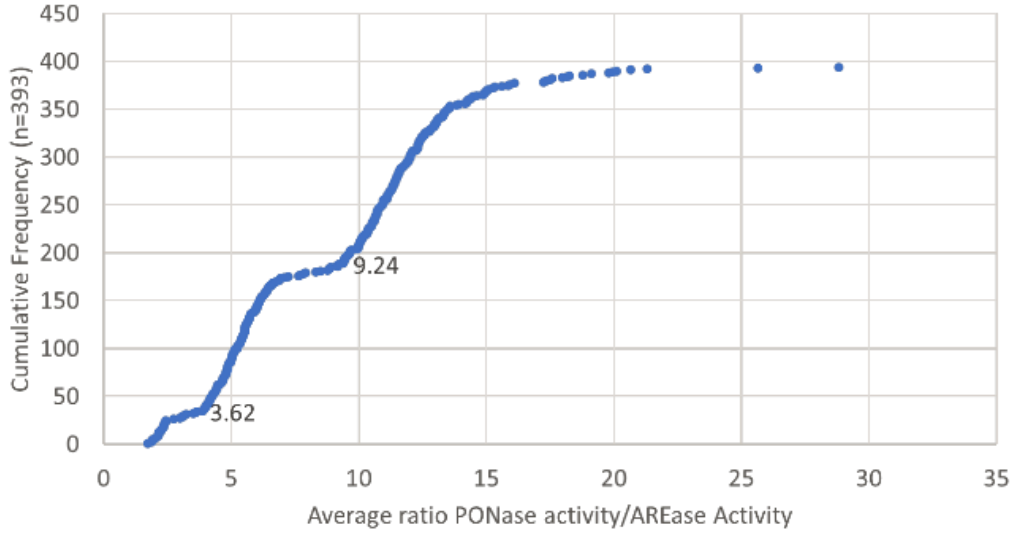
VIII. Figures

Figure 1: Chlorpyrifos Metabolism



Adapted from Timchalk et al. 2002

Figure 2: Cumulative Frequency Distribution for PON1 Phenotype Definition



The cumulative frequency of paraoxonase to arylesterase for each sample (n = 393 total samples).

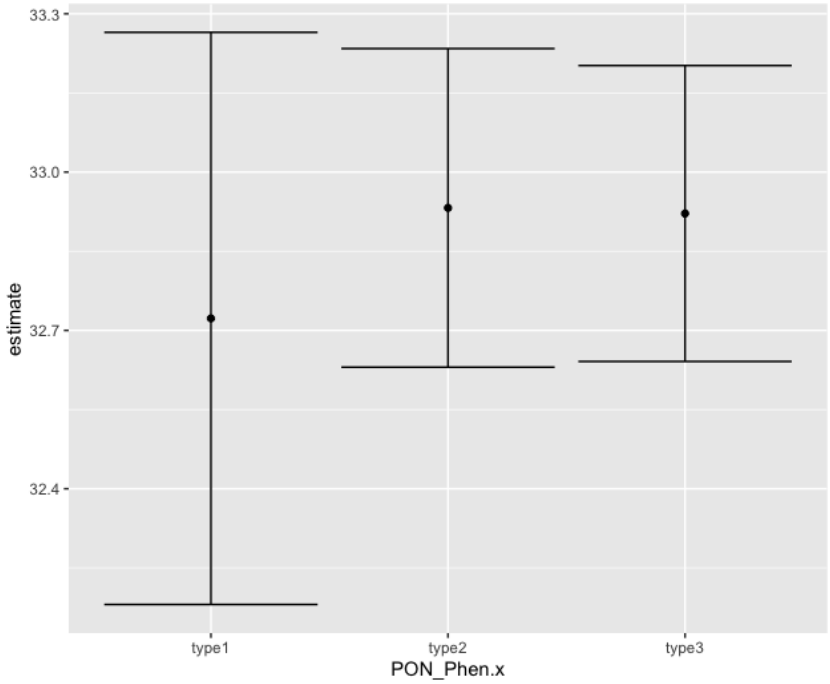
Average ratio of PONase activity/AREas activity:

Type 1 QQ Low metabolizers < 3.62

Type 2 QR Medium metabolizers ≥ 3.62 -< 9.24

Type 3 RR High metabolizers ≥ 9.24

Figure 3: Mean Head Circumference by PON1 Phenotype



Type 1: QQ low PON1 metabolizer
Type 2: QR medium PON1 metabolizer
Type 3: RR high PON1 metabolizer