ASSOCIATIONS BETWEEN MATERNAL EXPOSURES TO ORGANOPHOSPHATE AND PYRETHROID PESTICIDES AND PRETERM BIRTH USING DATA FROM THE ZIKA IN PREGNANCY (ZIP) COHORT STUDY

by

NICHOLAS MALLIS

(Under the Direction of José Cordero)

ABSTRACT

Background: Organophosphates (OP) and pyrethroids (PYR) are insecticides used worldwide in large scale farming, home applications, and insect repellents. Studies suggest that OP and PYR exposure during pregnancy are associated poor birth outcomes. The aims of this project are to 1) Compare OP and PYR metabolite levels among a diverse cohort of pregnant persons residing in Brazil, Guatemala, Nicaragua, and Puerto Rico and examine demographics that associate with OP exposure levels 2) Identify changes in metabolite exposure levels across gestation 3) Examine associations between organophosphate and pyrethroid pesticide biomarker levels and preterm birth (PTB). **Methods:** 1,326 pregnant persons were recruited from ZIP research sites in Puerto Rico, Nicaragua, Brazil, and Guatemala. Participants were first interviewed about demographic characteristics and followed throughout pregnancy until delivery. Urine samples were collected at regular visits and analyzed for pesticide biomarker concentrations. We measured seven organophosphate biomarkers (DEDP, DETP, DMDP, DMP, DMTP, and TCP) and three pyrethroid biomarkers (PBA, FPBA, PNP) **Results:** Aim 1) For OPs, participants from Brazil had the highest levels of TCP and DEP metabolites and Nicaragua had the highest DETP

levels. For pyrethroids, participants from Guatemala had the highest PNP levels and Nicaragua had the highest PBA levels. Primary water source, BMI category, education level, marital status, and locality were all significantly associated with organophosphate metabolite levels. Aim 2) Among the five metabolites examined in this study, TCP, was the only analyte that appeared to be increasing on average in the sample throughout gestation. Aim 3) OP and PYR exposure was not associated with an increased risk for preterm birth. Two pyrethroid metabolites (PBA and PNP) and one organophosphate metabolite (TCP) displayed a protective association against PTB. **Conclusions:** Our analysis adds to the growing body of knowledge on pesticide exposure during pregnancy. We found elevated levels in Brazil, higher levels than previous studies in the Americas, identified public drinking water as an exposure route to be further studied. Our analysis did not reveal OP or PYR exposure to be a risk factor for preterm birth.

INDEX WORDS: Pesticides, Preterm Birth, Pregnancy, Organophosphates, Pyrethroids, Birth Outcomes

ASSOCIATIONS BETWEEN MATERNAL EXPOSURES TO ORGANOPHOSPHATE AND PYRETHROID PESTICIDES AND PRETERM BIRTH USING DATA FROM THE ZIKA IN PREGNANCY (ZIP) COHORT STUDY

by

NICHOLAS MALLIS

BA, Florida State University, 2009

MPH, University of Georgia, 2019

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2023

ASSOCIATIONS BETWEEN MATERNAL EXPOSURES TO ORGANOPHOSPHATE AND PYRETHROID PESTICIDES AND PRETERM BIRTH USING DATA FROM THE ZIKA IN PREGNANCY (ZIP) COHORT STUDY

by

NICHOLAS MALLIS

Major Professor:	José Cordero
Committee:	Jessica Knight
	Ye Shen
	Travis Glenn

Electronic Version Approved:

Ron Walcott
Vice Provost for Graduate Education and Dean of the Graduate School
The University of Georgia
December 2023

ACKNOWLEDGEMENTS

Thanks to my committee, José Cordero, Jessica Knight, Ye Shen, and Travis Glenn for your guidance through this process. Thanks to Michael Welton for your collaboration in launching this project and collecting the data. Thanks to Skarlet Velasquez for your encouragement and insight. Thanks to Clarke Miller for your help with the unit conversions. Thanks to Ishaan Dave for your friendship, help, and encouragement through this process. Last but not least, thanks to my wife Carrie for your love, support, and encouragement.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION

Project Narrative

Organophosphates and Pyrethroids are common insecticides that came into prominence in the 1970's and were thought to be a safer option for pest control compared to organochlorines (DDT), which were banned in the US due to the deleterious effects on wildlife, persistence in the environment, and association with adverse health outcomes in humans (1). Organophosphates are mostly applied in large scale farming and structural pest control, while pyrethroids are used in large scale farming, home applications, and in insect repellents that are sprayed directly on clothes and mosquito nets (2). Common organophosphates pesticides include parathion, diazinon, malathion, and chlorpyrifos (2). Organophosphates poison insects and other pests by targeting acetylcholinesterase enzymes found in nerve endings, which causes organs to be overstimulated by excess acetylcholine and ultimately kills the insect via neurotoxicity (1). Permethrin, resmethrin, and sumithrin are common pyrethroids that are used around the world (2). Pyrethroids target the voltage-gated sodium channels in the nervous system in insects, causing paralysis and death (3). Both organophosphates and pyrethroids have been instrumental in advancing agriculture and public health efforts to combat vector borne diseases, but acute and long-term exposures have been associated with negative effects in humans (1).

Organophosphate exposure (acute and long term) can bring about negative health effects in the respiratory system (difficulty breathing, respiratory failure), cardiovascular system

(irregular or slow heartbeat), nervous system (neuropathy, memory loss, and paralysis), and gastrointestinal: (diarrhea, vomiting) (1). With pyrethroids, acute poisoning in humans can cause coughing and bronchospasm, nausea and vomiting (1). Humans can be exposed to small amounts of OPs and PYRs through food and water intake, inhalation, and direct contact (1). Individuals who work in agriculture and other occupations that regularly use pesticides are at a higher risk of exposure (4). While farmworkers and families living in close proximity to farming communities are at a higher risk for exposure to pesticides, it is also important to note that large quantity of the fruits, vegetables, and nuts humans consume contain trace amounts of OPs and PYRs, highlighting the importance of research on the topic (5, 6).

Several studies examining organophosphates and birth outcomes have found that OP exposure during pregnancy was associated with low birth weight, smaller head circumference, and smaller birth length (7-9). Existing literature also suggests that OP exposure during pregnancy is associated with neurodevelopmental and behavioral issues (10, 11). Less has been published on pyrethroids. A recent study published in 2022 in found that first and second trimester exposures to high levels of PYRs were associated with poorer infant neurodevelopmental outcomes by age one (12). Few studies have examined OPs and PYRs effects on preterm birth (PTB), the one of the outcomes of this analysis.

While the US has been moving to regulate and ban harmful pesticides, regulations have been less stringent in Latin American and South American countries, the regions in which our study population is located (13, 14). Agriculture makes up a large part of the economy in these regions and workers and residents may be at higher risk for exposure to pesticides (15). Characteristics associated with pesticide exposure among pregnant persons have been examined in different locations across the world, less has been published about the determinants of OP and

PYR pesticide exposure among pregnant persons in the Americas. Brazil, one of the sites in our present study, is among the top users of pesticides in the world and farming makes up a majority of the commerce in Guatemala and Nicaragua (16). It is important to examine these areas where there might higher levels of exposure. The Zika in Infants and Pregnancy Cohort launched in 2016 and was sponsored by the National Institutes of Health (NIH) and National Institute of Environmental Health Science. The ZIP cohort includes pregnant persons from multiple study sites in Brazil (4 sites), Columbia (1 site), Guatemala (1 site), Nicaragua (1 site), Puerto Rico (2 sites), and Peru (1 site). This study provided a unique opportunity to address knowledge gaps in the determinants of OP and PYR pesticide exposure and associations between exposure and pregnancy outcomes in the Americas.

Specific Aims

Aim 1: Compare organophosphate and pyrethroid distributions between countries in the Zika in Infants and Pregnancy (ZIP) Cohort, identify characteristics associated with urine organophosphate pesticide exposure among pregnant persons.

Aim 2: Examine changes in organophosphate and pyrethroid urine biomarker levels over time during pregnancy using data from the Zika in Infants and Pregnancy (ZIP) cohort.

Aim 3: Examine the association between organophosphate and pyrethroid urine biomarker levels during pregnancy and preterm birth using data from the Zika in Infants and Pregnancy (ZIP) cohort.

CHAPTER 2

LITERATURE REVIEW

Background on Organophosphates and Pyrethroids

Organophosphates (OPs) and pyrethroids (PYRs) are both insecticides widely used across the world. Organophosphates are mostly applied in large scale farming, while pyrethroids are used in large scale farming, home applications, and in insect repellents that are sprayed directly on clothes and mosquito nets (2). OPs and PYRs came into prominence in the 1970's and were thought to be a safer option for pest control compared to organochlorines (DDT), which were banned in the US due to the deleterious effects on wildlife, persistence in the environment, and association with adverse health outcomes in humans (1). Common organophosphates pesticides include parathion, diazinon, malathion, and chlorpyrifos (2). Organophosphates poison insects and other pests by targeting acetylcholinesterase enzymes found in nerve endings, which causes organs to be overstimulated by excess acetylcholine and ultimately kills the insect via neurotoxicity (1). Permethrin, resmethrin, and sumithrin are common pyrethroids that are used around the world (2). Pyrethroids target the voltage-gated sodium channels in the nervous system in insects, causing paralysis and death (3). Both organophosphates and pyrethroids have been instrumental in advancing agriculture and public health efforts to combat vector borne diseases, but acute and long-term exposures have been associated with negative effects in humans (1).

Humans can be exposed to small amounts of OPs and PYRs through food and water intake, inhalation, and direct contact (1). Individuals who work in agriculture and other

occupations that regularly use pesticides are at a higher risk of exposure (4). Similar to their intended effect on pests, OPs bring about toxic effects on humans by binding to the acetylcholinesterase enzyme and result in high levels of acetylcholine to build up. Both acute and long-term exposure can cause problems in the respiratory, cardiovascular, and nervous system (2). Acute exposure to OPs can result in experiencing diarrhea, vomiting, vision issues, paralysis, and even death (1). Studies on long term exposure to OPs have revealed neurologic effects and associations with neurological diseases (17, 18). Several studies examining organophosphate urine biomarkers have found that OP exposure during pregnancy was associated with low birth weight, smaller head circumference, and smaller birth length (7-9). Existing literature also suggests that OP exposure during pregnancy is associated with neurodevelopmental and behavioral issues (10, 11). With pyrethroids, acute poisoning in humans can cause coughing and bronchospasm, nausea and vomiting (1). Studies have also suggested that pyrethroids have adverse effects on male sperm concentration and motility (19, 20). A recent study published in 2022 in found that first and second trimester exposures to high levels of PYRs were associated with poorer infant neurodevelopmental outcomes by age one (12). Few studies have examined OPs and PYRs effects on preterm birth (PTB).

Background on Preterm Birth

Preterm birth is defined as babies who are born before 37 completed weeks of pregnancy (21). PTB is further classified into three different categories of preterm birth including extremely preterm (less than 28 weeks), very preterm (28 to 32 weeks), and moderate to late preterm (32 to 37 weeks). Preterm birth associated complications are the most common cause of death for children under 5 years old across the world (22). Short term complications can include lung,

heart, gastrointestinal, and immune system conditions which may lead to long-term complications that can include cerebral palsy, learning disabilities, vision and hearing problems, and chronic health issues (23). A 2012 report using data from 184 countries estimated the global average preterm birth rate to be 11.1% (9.1-13.4) (24). In the US, preterm birth rates have been steadily increasing since 2015, when the preterm rate was reported to be 9.6% (24). The most recent data from the National Center for Health Statistics reported the US preterm birth rate in 2022 to be 10.38% (25). In Puerto Rico, one of the sites in our study, preterm birth rates have also been on the rise. In 2021, they reported a 11.77% PTB rate and 12.03% in 2022, higher than the US national rate (25). Preterm birth is an outcome with multiple known factors including maternal factors, uterine, and fetal factors (26). Among maternal factors, Infections during pregnancy, maternal diabetes, hypertension, and other maternal conditions are common (26). Uterine restriction as in the case of twins or higher number multiple pregnancies is another known cause (26). Among fetal conditions, large for gestational age is an example. Placental conditions such as placenta previa may also lead to preterm birth. Still, for most preterm births, the cause is unknown (21)

Long term and acute exposure to chemicals such as pesticides have been of interest as possible factors that impact preterm birth. A comprehensive meta-analysis of pesticide exposure during pregnancy published in 2023 found moderate evidence of an association between pesticide exposure and preterm birth (27). Further, the research team found several studies that suggest second and third trimester exposures to be the critical windows in which there is an increased risk for PTB (27). Research suggests that pesticides might act as endocrine disruptors and bring about changes in metabolism and fetal growth during pregnancy, which could lead to preterm birth (28). Phthalates are one type of endocrine disruptor and exposure to phthalates

have been found to be associated with preterm birth and decreased gestation (29). Studies conducted among populations in Puerto Rico have found evidence that phthalate exposure during pregnancy is associated with preterm birth and mediated by testosterone, corticotrophin releasing hormone, and progesterone concentration levels (29). Further, they found these effects to only be apparent among males when stratifying by infant sex (29). Several studies using rats as subjects concluded that organophosphates prevent thyroid hormone-receptor binding, suggesting possible endocrine disrupting effects (30). In vitro studies using pyrethroid biomarkers also suggest that the metabolites have estrogenic effects and possible endocrine disruption (31). Recent studies have found that glyphosate herbicides (used in Roundup) are associated with preterm birth and decreased gestation (32). Glyphosates may act by inducing oxidative stress markers (8-iso-prostaglandin F2α and prostaglandin F2α) which have been found to be associated with preterm birth (33)

Organophosphates and Pyrethroids and Preterm Birth: Existing Studies

Studies that have examined the risk of OPs and/or PYRs exposure during pregnancy and PTB report diverse risk outcomes from no risk to an increased risk, and even some studies reporting exposure as protective. They also vary in study design, timepoint of data collection, and metabolites used to measure exposure, which makes drawing comparisons difficult. In 2022, Hu et. al. conducted an analysis using the China National Birth Cohort (n=302) and found that DEP urine concentration was associated with a higher risk of preterm birth (OR = 1.35, 95% CI = 1.11, 2.25) (34). Another cohort study in Bangladesh (n = 289) reported an increased risk for PTB among the high category 4-nitrophenol level as compared with the low (35). On the

other hand, a 2021 study in Argentina (n=776) found no association between organophosphate exposure and preterm birth (36).

Several studies examined associations between organophosphate levels and gestational age, without quantifying the relationship between OPs and PTB. In 2022, Rauch et. al. concluded that urinary DAP concentrations were associated with shorter gestation (n=344) (37). Eskenazi et. al. also reported an association between organophosphate levels and shortened gestation in their 2004 study (n= 488), specifically with samples collected later in pregnancy (10). While this study reported the association with gestational age as the outcome, the overall preterm birth rate was low in the cohort. In 2012, Wang et. al. reported that log unit increase in DEP was associated with shorter gestation (n=187), although the relationship was only apparent among females (20). Suwannakul et. al. conducted a study in Thailand in 2021 (n=71) and found a significant negative association with DEP levels and gestational age (38). Finally, a study conducted in Jordan in 2020 reported that DEDTP (n=104), an organophosphate biomarker, was negatively associated with gestational age (39).

Less studies have examined the relationship between pyrethroid biomarker concentrations and preterm birth. In 2020, Xu et. al. reported pyrethroid exposure to be significantly associated with a decreased risk for preterm birth (n=512) (40). A China based cohort study (n=545) conducted in 2015 found no association between pyrethroid biomarker concentration levels and preterm birth (41). Another study in Bangladesh (n=298) found no association between pyrethroid exposure and preterm birth (35).

Measuring Pesticide Levels in Humans Through Urine Metabolites

When describing what is known about the relationship between organophosphate and pyrethroid exposure and preterm birth, it is important to outline the methods in which pesticides are commonly measured in humans. When measuring pesticides, researchers commonly collect urine samples and test them for concentration levels of metabolite biomarkers produced after exposure to pesticides like OPs and PYRs. For example, Diethylphosphates are one of the six subtypes of Dialkylphosphates (DAPs), the metabolites used to assess organophosphate exposure in humans through urine samples. DEPs are not considered toxic themselves, but only regarded as biomarkers of OP pesticide exposure. It is estimated that close to 75% of OP pesticides are metabolized to dialkylphosphate metabolites that can be measured in humans, suggesting that not all of the exposure profile is always be captured(42). Further, measurement of DEPs and other DAPs are only thought to reflect recent exposure within a few days of the sample collection (43).

Organophosphate and Pyrethroid Levels Among Pregnant Populations

Studies around the world that have examined OP concentrations in pregnant persons have varying results. A cohort study conducted in California examined sum DEP and DETP concentration levels among 600 pregnant women residing in an agricultural community and reported a geometric mean of 23.9 ng/g (44). Another cohort based in Cincinnati, USA (n=327), reported a lower geometric mean sum DEP and DETP of 9.3 ng/g (45). Higher levels have been reported in other parts of the world. A cohort study in the Netherlands found geometric mean sum DEP/DETP level of 41.48 ng/g (46). Less studies have been conducted the region from which our study population was sourced. A 2014 study conducted in Puerto Rico (n=54) reported low geometric means for DEP and DETP (DEP GM = 0.9 ng/ml, DETP = 0.5 ng/ml) (47). These

concentration values were not corrected for urine dilution. The study from Puerto Rico also reported a percentage of samples above the LOD (DEP= 32.7%, DETP=51.3%). While less studies have been conducted on pregnant women in the regions in which our study sites were located, there have been examinations of organophosphate metabolite levels in other populations within these regions. One study conducted in Chile in 2011 among 190 children age 6-12 reported a geometric mean DEP level at 18.6 (1.09) ng/g creatinine and DETP at 3.7 (1.01) ng/g (48). Few studies have specifically examined predictors of pyrethroid urine metabolites among pregnant persons. In 2014, Dewailly et al published a descriptive analysis of pyrethroid biomarkers among 10 Caribbean countries and reported PBA geometric means ranging from 0.21 ng/l (Belize) to 1.77 ng/l (Antigua and Barbuda). They also reported the percent over LOD near 100% for all countries included for PBA. Using data from the PROTECT cohort from Puerto Rico (n=152), Lewis et al reported a 90% detection of PNP in the samples analyzed and calculated a geometric mean of 0.5 ng/ml.

Determinants of Organophosphate Exposure Among Pregnant Persons: Existing Studies

With growing knowledge on organophosphates and pyrethroids and their possible harmful effects on the fetus during pregnancy, it is important to consider demographics and characteristics that could be determinants of higher pesticide exposure in pregnant populations and identify regions with higher exposure levels. Existing studies on determinants of organophosphate pesticide metabolite levels among pregnant persons have displayed some common findings including elevated concentrations among those who consume higher amounts of fruits and vegetables (46, 49-51). A cohort study conducted in the Netherlands (n=784) in 2004 found increased levels in fruit consumption associated with higher metabolite

concentrations during pregnancy (46). This research team also reported higher levels among those who were in the normal or underweight BMI category vs overweight or obese and increases associated with higher age and higher education level (46). Another cohort study conducted in Spain reported similar findings, with increases in fruit and vegetable consumption associated with higher mean OP metabolite concentration levels among the 573 pregnant persons examined (49). They also reported higher levels among participants in lower BMI categories, who did not smoke cigarettes, and lived in an urban area as opposed to rural (49). Among a smaller group of pregnant persons in Puerto Rico (n=54), a 2015 study found increased levels of OP metabolites among those who reported consuming grape juice, peanuts, peanut butter, cherries, or raisins within two days of sample collection (50). This paper also reported significantly higher levels among younger participants, and those who were unemployed (50). Last, a larger group (n=247) or pregnant persons in Israel exhibited the same relationship between fruit consumption and increased dialkyl phosphate (DAP) concentration (51).

Changes in Pesticide Exposure Across Gestation: Existing Studies

Few studies have examined time trends across gestation with regard organophosphate and pyrethroids biomarkers, with some reporting increases across, some reporting decreases, and some reporting no change. One study conducted in the Netherlands did not see a change in DAP metabolite levels across gestation and reported similar median DAP metabolite levels in each trimester, which is consistent with the findings in our study (50). Another study specifically examined pyrethroid biomarkers and reported that PBA levels remined the same during pregnancy, but other pyrethroid biomarkers slightly increased (12). One study found sharp increases in organophosphate metabolites just after birth, but mean levels were similar during gestation (44). Another cohort study that examined endocrine disrupting chemicals (EDC) in

urine across gestation and reported that persistent EDCs tended to decrease across pregnancy time points (52).

Motivation and ZIP Cohort

While characteristics associated with pesticide exposure among pregnant persons have been examined in different locations across the world, less has been published about the determinants of OP and PYR pesticide exposure among pregnant persons in the Americas. Brazil, one of the sites in our present study, is among the top users of pesticides in the world and farming makes up a majority of the commerce in Guatemala and Nicaragua (16). It is important to examine these areas where there might higher levels of exposure. The Zika in Infants and Pregnancy Cohort launched in 2016 and was sponsored by the National Institutes of Health (NIH) and National Institute of Environmental Health Science. The ZIP cohort includes 6,100 pregnant persons from multiple study sites in Brazil (4 sites), Columbia (1 site), Guatemala (1 site), Nicaragua (1 site), Puerto Rico (2 sites), and Peru (1 site). Women were recruited in their first trimester or early second trimester and followed until delivery. Infants born to participants were also recruited into the ZIP study and followed for one year. This study provided a unique opportunity to address knowledge gaps about pesticides and pregnancy outcomes in the Americas.

CHAPTER 3

RESEARCH DESIGN AND METHODS

Study Population/Design

Our study includes participants who have been enrolled in the Zika in Infants and Pregnancy (ZIP) International Observational Cohort consortium, a large prospective cohort which aimed to provide a comprehensive analysis of maternal Zika infection and infant health outcomes. Investigation began in 2016 and is sponsored by the National Institutes of Health (NIH) and National Institute of Environmental Health Science. The ZIP cohort includes 6,100 pregnant persons from multiple study sites in Brazil (4 sites), Columbia (1 site), Guatemala (1 site), Nicaragua (1 site), Puerto Rico (2 sites), and Peru (1 site). Women were recruited in their first trimester or early second trimester and followed until delivery. Infants born to participants were also recruited into the ZIP study and followed for one year.

Women 15 years old or older who plan to give birth in the study regions and were not participating in other research studies were deemed eligible for the ZIP study. Participants were first interviewed in an initial visit for baseline demographics, medical history, and current pregnancy information. Participants then had monthly targeted clinical examinations where they were asked about occupational and household characteristics and other health behaviors. Biologic samples were collected at these visits including blood and urine, used for Zika virus (ZIKV) analysis and specimen repositories.

Our Sub-Study

1,326 mothers were recruited from ZIP research teams in Puerto Rico (North Karst), Nicaragua (Managua), Brazil (Salvador cite), and Guatemala (Guatemala City). For our study, additional urine samples aside from the ZIP study were collected at different time points during pregnancy. The samples were transported to the University of Georgia Center for Vaccine and Immunology Laboratory and stored in -20-degree freezers in 15-50 ml conical tubes. Our team sorted and aliquoted the samples into 5ml quantities before sending them to Mount Sinai's Children's Health Exposure Analysis Resource (CHEAR) for pesticide analysis.

CHEAR Pesticide Analysis

2,705 urine samples were sent to CHEAR and tested for organophosphate and pyrethroid pesticides. Six biomarkers, Diethyldithiophosphate (DEDP), Diethylphosphate (DEP), Diethylthiophosphate (DETP), Dimethyldithiophosphate (DMDP), Dimethylphosphate (DMP), Dimethylthiophosphate (DMTP), and 3,5,6-trichloro-2- pyridinol (TCP) were tested to detect organophosphate levels. Three biomarkers, 3-phenoxybenzoic acid (PBA), 4-fluoro-3-phenoxybenzoic acid (FPBA), and 4-nitrophenol (PNP)were tested to detect pyrethroid levels. The Limit of Detection (LOD) values reported were the average of the daily LODs for the whole study. The daily LODs were used to identify the valid values for each sample. Quality control pairs were used to measure of precision based on the percent of the difference between duplicate sample concentrations relative to the mean of the duplicate sample concentrations per subject. Creatinine levels were also tested for each urine sample and reported in mg/dL. These levels aid

in correcting for urine dilution when analyzing the data. In order to answer our research questions, we merged the lab data from the CHEAR urine analysis with epidemiologic data from the ZIP study.

Aim 1 Methods

In order to describe the study population, frequencies and percentages were calculated for each maternal characteristic. We calculated the percent over the limit of detection for each metabolite and stratified these results by site. We also plotted the percent over the limit of detection for each metabolite by site using stratified bar charts. Biomarkers with less than 70% Limit of Detection (LOD) were not included in the subsequent analysis, but differences percent detected by site were discussed in the results. In order to account for urine dilution in the samples, pesticide metabolites were corrected for creatinine level by dividing the concentration by the creatinine level before further analysis [creatinine corrected level = metabolite level concentration / creatinine concentration] (53). Among biomarkers that were included, samples with a concentration below the LOD were imputed using a standard formula in which the LOD is divided by the square root of two (54). For the metabolites with more than 70% detection, geometric means and standard deviations of urinary concentrations of pesticide metabolites were calculated and presented as a whole and stratified by site and maternal characteristics. Maternal characteristics include maternal education level, maternal age, marital status, smoking status, alcohol use, employment status, infant sex, locality, primary water source, and pre-pregnancy BMI category (underweight ≤ 18.5, healthy $18.5 < 24.9$, overweight $25 < 29.9$, obese ≥ 30). The frequency and percent of mothers missing measurements was tabulated and reported.

Results were reported for each biomarker individually and then these levels were added together for a sum DEP/DETP organophosphate exposure.

We used t-tests and ANOVA to compare creatinine corrected group means for each biomarker by levels of categorical variables. All tests were conducted at the alpha = 0.05 level. Tukey tests were used for pairwise comparisons. All tests used the log of the creatinine corrected biomarker value as the outcome.

For modeling, we used crude and adjusted linear regression models for each maternal characteristic and the creatinine corrected metabolite levels. For this analysis, we also used the log of the creatinine corrected metabolites as the outcome. We report beta coefficients, standard errors, and p-values for each covariate level. All tests were conducted at the alpha = 0.05 level. For variable selection, we used the least absolute shrinkage and selection operator (LASSO) method on each biomarkers to determine which variables were important in predicting the biomarker outcomes.

Aim 2 Methods

To display time trends visually, we plotted splines, which use a smoothing function to show the average trajectory of metabolite concentrations by gestational age in weeks. We present spline plots for DEP, DETP, PNP, PBA, and TCP. We also used ad hoc methods and stratified the splines by maternal characteristic levels in an attempt to pinpoint patterns. To further visualize possible relationships across time, we plotted boxplots by week of gestation for each biomarker, which helped visualize if certain metabolite levels tended to be higher at different points in pregnancy.

For modeling, we used crude and adjusted longitudinal models for each biomarker to quantify linear trends in metabolite levels across gestation, accounting for the correlation of repeated measurements. We also ran crude and adjusted models with a quadratic term to examine possible non-linear relationships with gestational age and metabolite levels. For all models, we used the log of the creatinine corrected biomarker. Models were adjusted for characteristics found to be significantly associated with the metabolites in aim 1. For DEP, characteristics included site and primary water source. For DETP, it included site, primary water source, BMI category, education level, and first pregnancy or not. For PNP, age category, BMI category, site, marital status, locality, water source, education level and employment status were included. For PBA, characteristics include site, BMI category, marital status, locality, water source, education level, smoking, and alcohol consumption and for TCP, we included site, marital status and education.

Aim 3 Methods

Exposure Ascertainment

Biomarkers with a limit of detection (LOD) greater than 70% were included in the study. For all organophosphate biomarkers, the LOD was 0.5 ng/mL. For all pyrethroid biomarkers, the LOD was 0.10 ng/mL. Out of all the OP biomarkers measured, DEP (98% detect), DETP (90% detect), and TCP (96% detect) had LODs greater than 70% and were therefore used as primary exposures in the analysis. Out of the PYR biomarkers measured PBA (90% detect) and PNP (96% detect) all had LODs greater than 70% and were used as the primary exposures in this analysis. DEDP (8% detect), DMDP (6% detect), DMP (56% detect), DMTP (49% detect), and FPBA (17% detect) were below the 70% and excluded from our analysis. Among biomarkers

that were included, samples with a concentration below the LOD were imputed using a standard formula in which the LOD is divided by the square root of two (54). Then, for each biomarker, concentration levels were divided by the creatinine level to correct for urine dilution [creatinine corrected level = metabolite level concentration / creatinine concentration] (53). Before modeling, we categorized each creatinine corrected biomarker into low, medium, and high categories using tertiles calculated for each metabolite. The biomarker category levels were used as the main exposures in the models.

Outcome Ascertainment

The outcome of the study, preterm birth, was defined as babies being born alive before 37 completed weeks of gestation. Gestational age at delivery was calculated based on last menstrual period and delivery date. One hundred and sixty-seven (12.5%) participants missing information on preterm birth status were removed before analysis.

Statistical Analysis

We calculated means, standard deviations, geometric means, geometric standard deviations and percentiles for each creatinine corrected metabolite (DEP, DETP, TCP, PBA, and PNP) stratified by preterm birth status. We used t-tests to compare group means for each biomarker by preterm birth status. Due to skewness in the data, t-tests were run on the log of each creatinine corrected biomarker outcome. Descriptive statistics on the study population were calculated and stratified by preterm birth. Maternal characteristics included study site, maternal education, maternal age, marital status, smoking status, alcohol use, employment status, infant sex, pre-pregnancy weight, and pre-pregnancy BMI. We conducted chi-square tests for

independence with each characteristic and the outcome. All tests were conducted at an alpha level of 0.05.

We calculated crude and adjusted logistic regression models for each creatinine corrected biomarker category level and preterm birth. The low level for each biomarker was used as the reference group. We present odds ratios and 95% confidence intervals for each biomarker category level. The adjusted models included site, age category, education level, pre-pregnancy BMI, marital status, smoking status, and sex of infant as covariates. Covariates were included in the adjusted model based upon prior knowledge of association with PTB, causal mapping, and maternal characteristics showing significant univariate associations with the outcome in our sample. In order to further explore associations, we also ran the models with gestational age at birth as the outcome.

CHAPTER 4

DETERMINANTS OF ORGANOPHOSPHATE AND PYRETHROID EXPOSURE AMONG THE ZIKA AND INFANTS IN PREGNANCY COHORT[1]

[1]Mallis, N., Welton, M.., Knight, J., Shen, Y., Glenn, T., Cordero, JF

To be submitted to Environment International

Abstract

Background: Organophosphates (OPs) and pyrethroids (PYRs) are insecticides used worldwide in large scale farming, home applications, and in insect repellents. Studies suggest that OP and PYR exposure during pregnancy are associated poor birth outcomes. Few studies in the Americas have examined determinants of OP and PYR metabolite levels among pregnant persons, and most existing studies source populations from the US mainland.

Objective: Our study aims to compare OP and PYR distributions between countries in the Zika in Infants and Pregnancy (ZIP) Cohort, identify characteristics associated with urine organophosphate pesticide exposure among pregnant persons, and compare distributions of metabolite levels to previous studies in populations of pregnant persons.

Methods: 1,326 pregnant persons were recruited from ZIP research sites in Puerto Rico (North Karst), Nicaragua (Managua), Brazil (Salvador), and Guatemala (Guatemala City). Participants were first interviewed about demographic characteristics and followed throughout pregnancy until delivery. Urine samples were collected at regular visits and analyzed for pesticide biomarker concentrations by Mount Sinai's Children's Health Exposure Analysis Resource (CHEAR). We measured seven OP biomarkers (DEDP), Diethylphosphate (DEP), Diethylthiophosphate (DETP), Dimethyldithiophosphate (DMDP), Dimethylphosphate (DMP), Dimethylthiophosphate (DMTP) and 3,5,6-trichloro-2- pyridinol (TCP). Three biomarkers, 3-phenoxybenzoic acid (PBA), 4-fluoro-3-phenoxybenzoic acid (FPBA), and 4-nitrophenol (PNP). We calculated geometric means for metabolites and stratified by country/site and maternal characteristics and calculated percent over limit of detection by site. We conducted t-tests and

ANOVA to compare levels by site and maternal characteristics. We used univariate and multivariable linear regression to calculate crude and adjusted beta coefficients for each maternal characteristic and the OP metabolites (DEP, DETP, and TCP). We used LASSO to select important predictors.

Results: There were significant differences by site for TCP, DEP, PNP and PBA levels. For TCP, Brazil had the highest geometric mean TCP level at 4.1 (1.68) ng/g ($p< 0.0001$). Nicaragua had the highest geometric mean DETP concentration (2.47 ng/g, $p < 0.0001$). For PNP, Guatemala had the highest geometric mean at 1.58 (0.04) ng/g ($p< 0.0001$). For PBA, Nicaragua had the highest geometric mean at 1.46 (0.06) ng/g ($p< 0.0001$). OP levels significantly varied by water source (higher in city water vs bottle/delivery for DEP and DETP) and locality (higher in downtown vs suburban for TCP). OP levels also significantly varied by the following personal characteristics: pre-pregnancy BMI (lower in obese vs normal for DEP and DETP), education level (higher in less than high school vs college for DETP), marital status (higher in those who are married or living with a partner vs single for TCP), and first pregnancy (higher in first pregnancy vs not for DETP). The LASSO model chose site, water source, and BMI as important predictors of OP exposure for DETP.

Conclusion/Discussion: For OPs, participants from Brazil had the highest levels of TCP and DEP metabolites and Nicaragua had the highest DETP levels. For pyrethroids, participants from Guatemala had the highest PNP levels and Nicaragua had the highest PBA levels. Primary water source, BMI category, education level, marital status, and locality were all significantly

associated with organophosphate metabolite levels. The geometric DEP an DETP mean levels in our study were higher than some previous studies conducted in the same regions, which could be explained by possible higher use of pesticides due to the Zika outbreak in 2015-2016.

Introduction

Organophosphates (OPs) and pyrethroids (PYRs) are both insecticides widely used across the world. Organophosphates are mostly applied in large scale farming, while pyrethroids are used in large scale farming, home applications, and in insect repellents that are sprayed directly on clothes and mosquito nets (2). OPs and PYRs came into prominence in the 1970's and were thought to be a safer option for pest control compared to organochlorines (DDT), which were banned in the US due to the deleterious effects on wildlife, persistence in the environment, and association with adverse health outcomes in humans (1). Common organophosphates pesticides include parathion, diazinon, malathion, and chlorpyrifos (2). Organophosphates poison insects and other pests by targeting acetylcholinesterase enzymes found in nerve endings, which causes organs to be overstimulated by excess acetylcholine and ultimately kills the insect via neurotoxicity (1). Permethrin, resmethrin, and sumithrin are common pyrethroids that are used around the world (2). Pyrethroids target the voltage-gated sodium channels in the nervous system in insects, causing paralysis and death (3). Both organophosphates and pyrethroids have been instrumental in advancing agriculture and public health efforts to combat vector borne diseases, but acute and long-term exposures have been associated with negative effects in humans (1).

Humans can be exposed to small amounts of OPs and PYRs through food and water intake, inhalation, and direct contact (1). Individuals who work in agriculture and other occupations that regularly use pesticides are at a higher risk of exposure (4). Similar to their intended effect on pests, OPs bring about toxic effects on humans by binding to the acetylcholinesterase enzyme and result in high levels of acetylcholine to build up. Both acute and long-term exposure can cause problems in the respiratory, cardiovascular, and nervous system (2). Acute exposure to OPs can result in experiencing diarrhea, vomiting, vision issues, paralysis,

and even death (1). Studies on long term exposure to OPs have revealed neurologic effects and associations with neurological diseases (17, 18). Several studies examining organophosphate urine biomarkers have found that OP exposure during pregnancy was associated with low birth weight, smaller head circumference, and smaller birth length (7-9). Existing literature also suggests that OP exposure during pregnancy is associated with neurodevelopmental and behavioral issues (10, 11). With pyrethroids, acute poisoning in humans can cause coughing and bronchospasm, nausea and vomiting (1). Studies have also suggested that pyrethroids have adverse effects on male sperm concentration and motility (19, 20). A recent study published in 2022 in found that first and second trimester exposures to high levels of PYRs were associated with poorer infant neurodevelopmental outcomes by age one (12). With growing knowledge on organophosphates and pyrethroids and their possible harmful effects on the fetus during pregnancy, it is important to consider demographics and characteristics that could be determinants of higher pesticide exposure in pregnant populations and identify regions with higher exposure levels.

Existing studies on determinants of organophosphate pesticide metabolite levels among pregnant persons have displayed some common findings including elevated concentrations among those who consume higher amounts of fruits and vegetables (46, 49-51). A cohort study conducted in the Netherlands (n=784) in 2004 found increased levels in fruit consumption associated with higher metabolite concentrations during pregnancy (46). This research team also reported higher levels among those who were in the normal or underweight BMI category vs overweight or obese and increases associated with higher age and higher education level (46). Another cohort study conducted in Spain reported similar findings, with increases in fruit and vegetable consumption associated with higher mean OP metabolite concentration levels among

the 573 pregnant persons examined (49). They also reported higher levels among participants in lower BMI categories, who did not smoke cigarettes, and lived in an urban area as opposed to rural (49). Among a smaller group of pregnant persons in Puerto Rico (n=54), a 2015 study found increased levels of OP metabolites among those who reported consuming grape juice, peanuts, peanut butter, cherries, or raisins within two days of sample collection (50). This paper also reported significantly higher levels among younger participants, and those who were unemployed (50). Last, a larger group (n=247) or pregnant persons in Israel exhibited the same relationship between fruit consumption and increased dialkyl phosphate (DAP) concentration (51).

While characteristics associated with pesticide exposure among pregnant persons have been examined in different locations across the world, less has been published about the determinants of OP and PYR pesticide exposure among pregnant persons in the Americas. Brazil, one of the sites in our present study, is among the top users of pesticides in the world and farming makes up a majority of the commerce in Guatemala and Nicaragua (16). It is important to examine these areas where there might higher levels of exposure. Our study aims to compare the distribution of organophosphate and pyrethroid metabolite levels between different countries/study sites and identify characteristics associated with metabolites of organophosphate pesticides among the Zika and Infants in Pregnancy Cohort.

Methods

Study Population

Our study includes participants who have been enrolled in the Zika in Infants and Pregnancy (ZIP) International Observational Cohort consortium, a large prospective cohort

which aimed to provide a comprehensive analysis of maternal Zika infection and infant health outcomes. Investigation began in 2016 and is sponsored by the National Institutes of Health (NIH) and National Institute of Environmental Health Science. The ZIP cohort includes 6,100 pregnant persons from multiple study sites in Brazil (4 sites), Columbia (1 site), Guatemala (1 site), Nicaragua (1 site), Puerto Rico (2 sites), and Peru (1 site). Women were recruited in their first trimester or early second trimester and followed until delivery. Infants born to participants were also recruited into the ZIP study and followed for one year.

Women 15 years old or older who plan to give birth in the study regions and were not participating in other research studies were deemed eligible for the ZIP study. Participants were first interviewed in an initial visit for baseline demographics, medical history, and current pregnancy information. Participants then had monthly targeted clinical examinations where they were asked about occupational and household characteristics and other health behaviors. Biologic samples were collected at these visits including blood and urine, used for Zika virus (ZIKV) analysis and specimen repositories.

Our Sub-Study

1,326 mothers were recruited from ZIP research teams in Puerto Rico (North Karst), Nicaragua (Managua), Brazil (Salvador cite), and Guatemala (Guatemala City). For our study, additional urine samples aside from the ZIP study were collected at different time points during pregnancy. The samples were transported to the University of Georgia Center for Vaccine and Immunology Laboratory and stored in -20-degree freezers in 15-50 ml conical tubes. Our team sorted and aliquoted the samples into 5ml quantities before sending them to Mount Sinai's Children's Health Exposure Analysis Resource (CHEAR) for pesticide analysis.

CHEAR Pesticide Analysis

2,705 urine samples were sent to CHEAR and tested for organophosphate and pyrethroid pesticides. Seven biomarkers, Diethyldithiophosphate (DEDP), Diethylphosphate (DEP), Diethylthiophosphate (DETP), Dimethyldithiophosphate (DMDP), Dimethylphosphate (DMP), Dimethylthiophosphate (DMTP), and 3,5,6-trichloro-2- pyridinol (TCP) were tested to detect organophosphate levels. Three biomarkers, 3-phenoxybenzoic acid (PBA), 4-fluoro-3-phenoxybenzoic acid (FPBA), and 4-nitrophenol (PNP)were tested to detect pyrethroid levels. The Limit of Detection (LOD) values reported were the average of the daily LODs for the whole study. The daily LODs were used to identify the valid values for each sample. Quality control pairs were used to measure of precision based on the percent of the difference between duplicate sample concentrations relative to the mean of the duplicate sample concentrations per subject. Creatinine levels were also tested for each urine sample and reported in mg/dL. These levels aid in correcting for urine dilution when analyzing the data. In order to answer our research questions, we merged the lab data from the CHEAR urine analysis with epidemiologic data from the ZIP study. For this analysis, we used the first sample collection for each of the participants in our study. Further analysis in aims 2 examines how distributions change across gestation and aim 3 examines distributions of samples taken later in pregnancy.

Statistical Analysis

In order to describe the study population, frequencies and percentages were calculated for each maternal characteristic. We calculated the percent over the limit of detection for each metabolite and stratified these results by site. We also plotted the percent over the limit of detection for each metabolite by site using stratified bar charts. Biomarkers with less than 70%

Limit of Detection (LOD) were not included in the subsequent analysis, but differences percent detected by site tabulated and discussed in the results. In order to account for urine dilution in the samples, pesticide metabolites were corrected for creatinine level by dividing the concentration by the creatinine level before further analysis [creatinine corrected level = metabolite level concentration / creatinine concentration] (53). Among biomarkers that were included, samples with a concentration below the LOD were imputed using a standard formula in which the LOD is divided by the square root of two (54). For the metabolites with more than 70% detection, geometric means and standard deviations of urinary concentrations of pesticide metabolites were calculated and presented as a whole and stratified by site and maternal characteristics. Maternal characteristics include maternal education level, maternal age, marital status, smoking status, alcohol use, employment status, infant sex, locality, primary water source, and pre-pregnancy BMI category (underweight ≤ 18.5, healthy $18.5 < 24.9$, overweight $25 < 29.9$, obese ≥ 30). The frequency and percent of mothers missing measurements was tabulated and reported. Results were reported for each biomarker individually and then sum of the two DAP metabolites were added together for sum DEP/DETP exposure to compare to other studies in the discussion.

We used t-tests and ANOVA to compare creatinine corrected group means for each organophosphate biomarker by levels of categorical variables. All tests were conducted at the alpha = 0.05 level. Tukey tests were used for pairwise comparisons. All tests used the log of the creatinine corrected metabolite as the outcome. We used univariate linear regression models for each maternal characteristic and our creatinine corrected organophosphate biomarker levels. For this analysis, we also used the log of the creatinine corrected organophosphate biomarker values as the outcome. We report beta coefficients, standard errors, and p-values for each covariate level. All tests were conducted at the alpha = 0.05 level. For each organophosphate biomarker,

we ran adjusted models controlling for all maternal characteristics and report beta coefficients, standard errors, and p-values for each covariate level. For variable selection, we used the least absolute shrinkage and selection operator (LASSO) method on each biomarkers to determine which variables were important in predicting the biomarker outcomes.

Results

The distribution of the study population characteristics is presented in Table 4.1. The majority of the participants were under 25 years of age (54.22%), in the healthy BMI category (45.32%), and came from the Guatemala study site (37.03%). Most of the participants were either married or living with a partner (84%) and currently unemployed (69%). Forty-five percent of the participants did not have a high school diploma.

Organophosphate Descriptive Statistics by Site

Diethylphosphates (DEPs) were detected in 98% of the study samples and Diethyliophosphates (DETPs) were detected in 90%. DEDP (8.7% detect), DMDP (6% detect), DMP (48.27% detect), and DMTP (44.57% detect) were below the 70% (Table 4.2). Ninety two percent were over the LOD for TCP. When examining the percent over limit of detection by site, we see that Puerto Rico had the highest over the limit of detection for DEDP (15.32%), DEP (98.84%), and DMDP (7.8%) (Table 4.3, Figure 4.1). Brazil had the highest percent over the limit of detection for DETP (93.2%), DMP (61.2%), and DMTP (63.27%). Eighty-eight percent of the samples were above the LOD for both diethylphosphate (DEP) and dielthyliophosphate (DETP), showing that on average, when one organophosphate biomarker was present, the other was also present. Percent of samples greater than the limit of detection for TCP also varied by

site. Brazil had the highest (99.32%) percent greater than the limit of detection for TCP and Puerto Rico had the lowest (83.53%).

For the total population, the geometric means (SE) were 17.06 (0.6) ng/g creatinine for DEP and 1.9 (0.05) ng/mg creatinine for DETP (Table 4.2). The geometric mean for TCP was 1.16 (0.06) ng/g creatinine. Participants from Brazil had the highest levels of DEP concentrations [GM(SD) = 19.9 (1.4) ng/g creatinine] and Puerto Rico had the lowest [GM(SD) = 16.4 (1.0) ng/g creatinine], but these differences were not significant at the 0.05 level (p=0.24) (Table 4.5). Nicaragua had the highest geometric mean DETP concentration (2.47 ng/g) and Puerto Rico had the lowest (1.45 ng/g, $p < 0.0001$). For TCP, Brazil had the highest geometric mean at 4.1 (1.68) ng/g and Puerto Rico had the lowest at 0.26 (0.024) ng/g ($p< 0.0001$).

Pyrethroid Descriptive Statistics by Site

For the whole group used in this analysis, 96.2% of samples were over the limit of detection (LOD) for PNP, 86% were over the LOD for PBA and only 15.46% were over the LOD for FPBA (Table 4.2). When examining the percent greater than the limit of detection by site, we see that all PNP percentages were around the same (95.52-96.6%) for each site (Table 4.4). The percent of samples greater than the limit of detection for PBA varied by site (Table 4.4, Figure 4.2). Brazil (96.94%) had the highest percent greater than the limit of detection for PBA and Guatemala had the lowest (70.06%). Puerto Rico had the highest percent of samples over the limit of detection for FPBA (16%).

PNP had the highest geometric mean (SD) level for the whole population compared with other metabolites at 1.197 (0.02) ng/g creatinine (Table 4.2). The geometric mean for PBA was 0.82 (0.03) ng/g creatinine. For each metabolite, ANOVA tests revealed significant differences

in group geometric means by site (table 6). For PNP, Guatemala had the highest geometric mean at 1.58 (0.04) ng/g and Puerto Rico had the lowest at 0.9 (0.04) ng/g ($p< 0.0001$). For PBA, Nicaragua had the highest geometric mean at 1.46 (0.06) ng/g and Guatemala had the lowest at 0.378 (0.024) ng/g ($p< 0.0001$).

Maternal Characteristics and Organophosphates

There were significantly higher creatinine corrected DEP levels in those who reported their primary water source to be city water vs bottle delivery (18.5 ng/g vs 14.48 ng/g, $p= 0.04$) (Table 4.7). For all other covariates, t-tests and ANOVA did not reveal significant differences with regard to creatinine corrected DEP levels. We detected significantly higher creatinine corrected DETP levels in those who reported their primary water source to be city water vs bottle delivery (2.07 ng/g vs 1.65 ng/g, $p= 0.0002$). Several other covariates displayed significant differences with regard to DETP concentrations. DETP levels were higher among those in the underweight BMI category vs overweight (2.0 ng/g vs 1.84 ng/g, $p <0.0001$) and higher in the underweight BMI category vs obese (2.0 ng/g vs 1.48 ng/g, $p <0.0001$). DETP concentration levels were lower among those who attended college vs those with a high school diploma or less (1.56 ng/g vs 2 ng/g, $p < 0.0001$). DETP levels were also significantly higher in those who reported that it was their first pregnancy vs not first pregnancy (2.06 ng/g vs 1.81 ng/g, $p = 0.01$).

For TCP, t tests and ANOVA tests revealed significant differences in group geometric means by marital status and education level (table 4.8). Those who were married (GM=1.4 ng/g) had higher TCP levels compared with those who were single (GM=0.85 ng/g) ($p=0.0032$). Participants with less than a high school diploma had the highest TCP levels (GM=1.44 ng/mg)

vs those with a high school diploma (GM=1.36 ng/g) or a college degree (0.61 ng/mg) ($p < 0.0001$).

The crude models with log DEP concentrations as the outcome showed on average a significantly lower concentration among participants who reported their primary water source to be bottled water vs city water [Beta (SE) = -0.18 (0.08), p = 0.018] (Table 4.9). This association was not significant in the adjusted model. The univariate model with site and DEP as the outcome revealed significantly higher mean DEP levels among participants from Brazil compared with Puerto Rico [Beta(SE) = 0.20 (0.10), p = 0.046]. The LASSO model used to choose important predictors did not select any of the maternal characteristics as important in predicting DEP concentrations.

The crude and adjusted models with log DETP concentration as the outcome showed on average a significantly lower concentration among participants who reported their primary water source to be bottled water vs city water [Adjusted Beta (SE) = -0.28 (0.07), p <0.0001] Table 4.10). There were also significant differences between among those who were in the obese pre-pregnancy BMI category vs healthy weight [Adjusted Beta (SE) = -0.26 (0.08), p =0.0009] . The adjusted models with DETP as the outcome revealed higher concentrations among Guatemala vs Puerto Rico [Adjusted Beta (SE) = 0.35 (0.09), p = 0.0003] and Nicaragua vs Puerto Rico [Adjusted Beta (SE) = 0.37 (0.11), p = 0.0006]. The adjusted model also revealed lower concentrations in the obese BMI category vs the healthy category [Adjusted Beta (SE) = -0.26 (0.08), p =0.0009] and lower among those who reported that it was not their first pregnancy [Adjusted Beta (SE) = -0.16 (0.07), p =0.017]. The crude model with locality and DETP revealed significantly higher creatinine corrected DETP concentrations among participants who reported living in downtown areas vs suburban areas [Beta (SE) = 0.11 (0.05), p <0.039], but these

differences were not detected in the adjusted model. In the crude model with education level and DETP concentration, those who reported completing some college had lower levels compared with those with less than a high school diploma [Beta (SE) = 0.25 (0.06), p <0.0001], but this difference was not significant in the adjusted model. The LASSO model with DETP as the outcome chose site, water source, and BMI category as important predictors.

The crude models with log TCP as the outcome revealed differences by site (higher in sites vs Puerto Rico), marital status (higher among living together or married), and water source (higher in well water vs city) (table 4.10). In the adjusted model with log TCP as the outcome, Brazil, Guatemala, and Nicaragua were all significantly higher than Puerto Rico, (p<0.001) and no other variables were significant. The LASSO model only chose site as an important variables in predicting log TCP levels.

Discussion

Our analysis revealed significant differences in organophosphates and pyrethroid metabolite levels by site in the Zika and Infants in Pregnancy Cohort. Participants from Brazil had the highest geometric mean levels of TCP and DEP metabolites and Nicaragua had the highest DETP levels. For pyrethroids, participants from Guatemala had the highest PNP levels and Nicaragua had the highest PBA levels. OP levels significantly varied by water source (higher in city water vs bottle/delivery for DEP and DETP and higher in well water vs city water for TCP) and locality (higher in downtown vs suburban for TCP). OP levels also significantly varied by the following personal characteristics: pre-pregnancy BMI (lower in obese vs normal for DEP and DETP), education level (higher in less than high school vs college for DETP), marital status (higher in those who are married or living with a partner vs single for TCP), and first pregnancy

(higher in first pregnancy vs not for DETP). The LASSO model chose site, water source, and BMI as important predictors of OP exposure (DETP).

Other studies have also found associations between primary water source water and OP metabolite levels. A 2020 study conducted in Thailand examined characteristics associated with OP biomarker levels among farmworkers both male and female (n=100) (55). The research team found significantly higher DEP concentrations among those who report tap water as their primary source compared with those who reported bottled water and they also concluded water source to be the most important predictor in their analysis of DEP and DETP concentrations (55). Runoff from pesticide use in agriculture and home applications can transfer to surface and ground water treated for drinking use (56). A 2021 study conducted in Costa Rica examined pesticide occurrence and water quality and found detections of chlorpyrifos, an organophosphate pesticide, in both water and sediment samples (57). They identified chlorpyrifos and other pesticides as possible factors in lower water quality in microcatchments near the sample collections (57).

Previous research on organophosphate biomarkers has also found a protective association among those who are overweight or obese. A study published in 2018 using cohort of pregnant women (n=784) in the Netherlands found higher levels of organophosphate metabolite concentration among the group in the normal weight or underweight categories (46). A similar 2016 study conducted in Canada on associations between maternal characteristics and organophosphate biomarkers levels also revealed lower metabolite levels among those in the high pre-pregnancy BMI category compared with normal (58). Studies suggest that those in higher BMI categories might expel creatinine at a higher rate, which could cause their creatinine corrected metabolite levels to be lower (59). Other studies have reported education to be a

determinant of OP levels among pregnant persons, but in these analyses higher education was associated with higher levels, which was different from our finding (38).

Other studies around the world that have examined sum DEP and DETP concentrations in pregnant persons have varying results. A cohort study conducted in California examined sum DEP and DETP concentration levels among 600 pregnant women residing in an agricultural community and reported a geometric mean of 23.9 ng/g, slightly higher than the findings in our study (44). Another cohort based in Cincinnati, USA (n=327), reported a lower geometric mean sum DEP and DETP of 9.3 ng/g (45). Higher levels have been reported in other parts of the world. A cohort study in the Netherlands found geometric mean sum DEP/DETP level of 41.48 ng/g (46). Less studies have been conducted the region from which our study population was sourced. A 2014 study conducted in Puerto Rico (n=54) reported geometric means for DEP and DETP much lower than the geometric means in our study (DEP GM = 0.9 ng/ml, DETP = 0.5 ng/ml) (47). These concentration values were not corrected for urine dilution. In our study, the uncorrected geometric means for the whole population were 12.08 ng/ml for DEP and 1.29 ng/ml for DETP. Among the participants in our study from Puerto Rico (n=346) the uncorrected geometric means were 15.13 ng/ml for DEP and 1.34 ng/ml for DETP. The study from Puerto Rico also reported a lower percentage of samples above the LOD (DEP= 32.7%, DETP=51.3%). The reason for these vast differences in DEP and DETP concentration levels among those in our study could be attributed to the fact that our participants had urine collections during the 2015-2016 zika outbreak, a vector borne disease that was occurring at high rates in central and south America. Infants of pregnant persons infected with zika can be born with microcephaly. Participants in our study resided in Brazil, Guatemala, Nicaragua, and Puerto Rico, all places in which zika was a public health issue at the time of data collection (60). People residing in these

regions might have used pesticides at higher rates than usual. While less studies have been conducted on pregnant women in the regions in which our study sites were located, there have been examinations of organophosphate metabolite levels in other populations within these regions. One study conducted in Chile in 2011 among 190 children age 6-12 reported a geometric mean DEP level at 18.6 (1.09) ng/g creatinine and DETP at 3.7 (1.01) ng/g, both of which were similar to our study (48).

Few studies have specifically examined predictors of pyrethroid urine metabolites among pregnant persons. In 2014, Dewailly et al published a descriptive analysis of pyrethroid biomarkers among 10 Caribbean countries and reported PBA geometric means ranging from 0.21 ng/l (Belize) to 1.77 ng/l (Antigua and Barbuda). They also reported the percent over LOD at 100% for all countries included for PBA. The geometric mean levels in our study were similar to these findings (GM=0.82 ng/g), but our percent over LOD for PBA was 86% for the whole sample, lower than the previous findings. Using data from the PROTECT cohort from Puerto Rico (n=152), Lewis et al reported a 90% detection of PNP in the samples analyzed and calculated a geometric mean of 0.5 ng/ml. These values were not corrected for urine dilution. When examining the uncorrected geometric means for PNP in our study, we see that the geometric mean for PNP in the whole sample was 0.81 ng/ml in among those from Puerto Rico it was 0.84 ng/ml, both of which are higher than the study mentioned above.

DEP, DETP, and TCP metabolites are biomarkers for exposure to chlorpyrifos, an organophosphate insecticide used in agriculture and structural pest control. Chlorpyrifos, has been associated with delays in psychomotor and mental development and ADHD in children and is now banned for home use in the US (61). Other less common organophosphates from which DEP and DETP metabolites are also biomarkers of include chlorethoxyphos, coumaphos,

disulfoton, ethion, phorate, sulfotepp, and terbufos. TCP is also a biomarker triclopyr, a herbicide used in home lawns and gardens.

We present a comprehensive exploratory analysis of maternal characteristics and their associations with urine metabolite biomarkers using a large, diverse cohort, but there are some limitations to consider when interpreting the results. First, data was not available on certain covariates that would have been of interest. Information on dietary (fruit and vegetable) intake, type of occupation (agriculture vs other types of employment), and pesticide usage habits would have been helpful in the analysis and interpretation of the results. Several studies have revealed positive associations between fruit and vegetable intake and urine organophosphate pesticide biomarkers, as well as increased levels among those who work in agriculture. Measuring urine biomarker levels during pregnancy provides an indication of pesticide exposure, but it is important to note that these measurements reflect recent exposures that would have occurred within a few days of the sample collection (43). Our sample collections might not have detected high exposures that could have occurred several days or weeks after. Last, while we were able to obtain information on a large sample of pregnant persons from four different countries, the results might not be generalizable to other populations outside of the Americas. When analyzing urine metabolites, it is difficult to identify the specific insecticide that the human subject has been exposed to because metabolites be biomarkers for several different types of pesticides and chemicals.

Given the associations revealed between water source and OP metabolite levels, we recommend future studies that not only test for urine levels in the subjects, but also in the source from which they draw their water for drinking and cooking. Comparisons between subject metabolite levels and levels in the water between groups could reveal more information about

this association. Future studies conducted on maternal characteristics associated with pesticide exposure should also include dietary intake information on participants and pesticide usage habits that will likely impact urine biomarker levels.

Table 4.1 Maternal Characteristics of Population

Characteristic	**Total (n=1,326)**
Age Category in Years, N(%)	
<25	719 (54.22)
25-30	366 (27.6)
>30	238 (17.95)
Missing	3 (0.23)
Pre-Pregnancy BMI Category (Kilograms/Height^2)	
Underweight ($\leq$ 18.5)	133 (10.03)
Healthy Weight (18.5 < 24.9)	601 (45.32)
Overweight (25 < 29.9)	338 (25.49)
Obese($\geq$ 30)	237 (17.87)
Missing	17 (1.28)
Site	
Brazil-Salvador	294 (22.17)
Guatemala	491 (37.03)
Nicaragua	195 (14.7)
Puerto Rico-North Karst	346 (26.09)
Marital Status	
Single	211 (15.91)
Married	404 (30.47)
Living Together, Not Married	704 (53.09)
Missing	7 (.053)
Education Level	
Less than a High School Diploma	609 (45.9)
High School Diploma or Equivalent	416 (31.37)
Associate Degree, Bachelor's Degree or Higher	296 (22.32)
Missing	5 (.38)
Employment Status	
Currently Employed	307 (23.15)
Not Currently Employed	923(69.61)
Missing	96 (6.9)
Smoking Status	
Current Smoker	10 (0.75)
Former Smoker	111 (8.37)
Never Smoked	1,045 (78.81)
Missing	160 (12.07)
First Pregnancy	
First Pregnancy	501 (37.78)
Not First Pregnancy	820 (61.84)
Missing	5 (0.38)
Sex of Infant	
Female	553 (41.7)
Male	602 (45.3)
Missing	171 (12.9)

Table 4.2. Distribution of Organophosphate and Pyrethroid Metabolite Concentrations Corrected for Creatinine (n= 1,326)

Metabolite	**% Detect**	**Geometric Mean (SE) in ng/g creatinine**
OP Metabolites		
DEP	98%	17.7 (0.6)
DETP	90%	1.9 (0.05)
DEP+DETP	NC	20.6 (0.06)
DEDP	8.7%	0.13 (0.0004)
DMDP	6%	0.12 (0.003)
DMP	48.27%	0.39 (0.02)
DMTP	44.57%	0.31 (0.02)
TCP	92.1%	1.16 (0.06)
PYR Metabolites		
PNP	96.2%	1.197 (0.02)
PBA	86%	0.82 (0.03)
FPBA	15.46%	0.04 (0.001)

Diethyldithiophosphate (DEDP), Diethylphosphate (DEP), Diethylthiophosphate (DETP), Dimethyldithiophosphate (DMDP), Dimethylphosphate (DMP), Dimethylthiophosphate (DMTP), 3-phenoxybenzoic acid (PBA), 4-fluoro-3-phenoxybenzoic acid (FPBA), 4-nitrophenol (PNP), and 3,5,6-trichloro-2- pyridinol (TCP)

Table 4.3. Percent of Samples Greater than the Limit of Detection (LOD) for all Organophosphate Metabolites by Site

Site	%Detect DEDP	%Detect DEP	% Detect DETP	% Detect DMDP	% Detect DMP	% Detect DMTP	%Detect TCP
Brazil	9.86%	98.64%	93.2%	5.1%	61.2%	63.27%	99.32%
Guatemala	4.28%	96.13%	86.35%	5.91%	28.1%	35.23%	93.48%
Nicaragua	6.15%	96.92%	91.28%	4.62%	31.79%	34.87%	92.82%
Puerto Rico	15.32%	98.84%	90.75%	7.8%	53.2%	47.4%	83.53%

Diethyldithiophosphate (DEDP), Diethylphosphate (DEP), Diethylthiophosphate (DETP), Dimethyldithiophosphate (DMDP), Dimethylphosphate (DMP), Dimethylthiophosphate (DMTP), 3,5,6-trichloro-2- pyridinol (TCP)

Table 4.4. Percent of Samples Greater than the Limit of Detection (LOD) for all Pyrethroid Metabolites by Site

Site	%Detect FPBA	%Detect PBA	%Detect PNP
Brazil	16%	96.94%	96.60%
Guatemala	10.6%	70.06%	95.52%
Nicaragua	12.3%	95.90%	96.41%
Puerto Rico	23.7%	94.22%	96.53%

3-phenoxybenzoic acid (PBA), 4-fluoro-3-phenoxybenzoic acid (FPBA), 4-nitrophenol (PNP)

Table 4.5. Distribution of Organophosphate Metabolite Concentrations by Site (n=1,326)

Site	**DEP Geometric Mean (SE) ***	**DETP Geometric Mean (SE)***	**SUM DEP/DETP Geometric Mean (SE)***	**TCP Geometric Mean (SE) ***
Brazil	19.9 (1.4)	1.87 (0.10)	23.7 (1.4)	4.10 (1.68)
Guatemala	17.34 (1.04)	2.08 (0.08)	21.9 (0.98)	1.59 (0.11)
Nicaragua	18.04 (1.62)	2.47 (0.16)	22.6 (1.7)	1.1 (0.13)
Puerto Rico	16.4 (1.0)	1.45 (0.07)	19.1 (0.98)	0.26 (0.024)
	p-value =0.24	**p < .0001**	**p = 0.0368**	**p <.0001**

Diethyldithiophosphate (DEDP), Diethylphosphate (DEP), Diethylthiophosphate, 3,5,6-trichloro-2- pyridinol (TCP)
**corrected for creatinine*

Table 4.6. Distribution of Pyrethroid Metabolite Concentrations by Site (n=1,326)

Site	**PNP Geometric Mean (SE)***	**PBA Geometric Mean (SE)***
Brazil-Salvador	0.98 (0.03)	1.32 (0.06)
Guatemala	1.58 (0.04)	0.378 (0.024)
Nicaragua	1.3 (0.04)	1.46 (0.11)
Puerto Rico-North Karst	0.90 (0.04)	1.2 (0.08)
	p < .0001	**p <.0001**

3-phenoxybenzoic acid (PBA), 4-nitrophenol (PNP)
**creatinine corrected*

Table 4.7 DEP and DETP Metabolite Concentrations by Maternal Characteristics (n=1,327)

Characteristic	DEP GEOMETRIC MEAN (SD) ng/g creatinine	p-value	DETP GEOMETRIC MEAN (SD) ng/g creatinine	p-value
Age Category in Years		p = 0.81		p = 0.11
<25	17.56 (0.76)		1.99 (0.07)	
25-30	17.45 (1.27)		1.82 (0.08)	
>30	18.57 (1.49)		1.76 (0.11)	
Site		p = 0.24		**p < 0.0001*** **
Brazil-Salvador	19.9 (1.4)		1.87 (0.10)	
Guatemala	17.34 (1.04)		2.08 (0.08)	
Nicaragua	18.04 (1.62)		2.47 (0.16)	
Puerto Rico-North Karst	16.4 (1.0)		1.45 (0.07)	
Locality		p= 0.34		p = 0.81
Downtown	16.6 (1.07)		2.04 (0.09)	
Suburban	17.9 (0.78)		1.83 (0.06)	
Water Source		**p = 0.04*** **		**p = 0.0002*** **
Town/City Water	18.53 (0.89)		2.07 (0.07)	
Bottled/Delivered Water	14.48 (0.95)		1.65 (0.07)	
Well Water	18.1 (2.27)		1.94 (0.18)	
Pre-Pregnancy BMI in Kilograms/Height^2		p = 0.2		**p <0.0001*** **
Underweight ($\leq$ 18.5)	18.84 (1.84)		2.0 (0.16)	
Healthy Weight (18.5 < 24.9)	18.65 (0.97)		2.09 (0.08)	
Overweight (25 < 29.9)	17.6 (1.2)		1.84 (0.09)	
Obese($\geq$ 30)	15.3 (1.3)		1.48 (0.08)	
Marital Status		p = 0.57		p = 0.30
Single	19.26 (1.32)		1.74 (0.11)	
Married	17.48 (1.02)		1.94 (0.09)	
Living Together, Not Married	17.4 (0.89)		1.92 (0.06)	
Education Level		p = 0.56		**p < 0.0001*** **
Less than a High School Diploma	17.36 (0.91)		2 (0.07)	
High School Diploma or Equivalent	18.7 (1.36)		2.03 (0.08)	
Associate Degree, Bachelor's Degree or Higher	17.13 (1.55)		1.56 (0.08)	
Employment Status		p = 0.26		p = 0.43
Currently Employed	19.11 (1.32)		1.96 (0.1)	
Not Currently Employed	16.9 (0.71)		1.87 (0.05)	
Smoking Status		p = 0.87		p = 0.1021
Current Smoker	20.7 (5.5)		1.06 (0.31)	
Former Smoker	16.9 (2.16)		1.95 (0.19)	
Never Smoked	17.5 (0.67)		1.94 (0.05)	
Alcohol Consumption Status		p = 0.47		p = 0.06
Current Drinking	19.62 (1.71)		1.69 (0.13)	
Before Pregnancy	16.73 (1.68)		1.75 (0.12)	
Does Not Drink	17.33 (0.73)		1.96 (0.06)	
First Pregnancy		p = 0.91		**p = 0.0103*** **
First Pregnancy	17.8 (0.96)		2.06 (0.08)	
Not First Pregnancy	17.6 (0.78)		1.81 (0.06)	
Sex of Infant		p = 0.72		p = 0.8441
Female	17.53 (0.94)		1.93 (0.07)	
Male	17.08 (0.9)		1.91 (0.07)	

*** statistically significant at the alpha = 0.05 level

Table 4.8 TCP Metabolite Concentrations by Maternal Characteristics (n=1,327)

Characteristic	TCP Geometric Mean (SE) in ng/g creatinine	p-value
Age Category in Years		p = 0.1753
<25	1.13 (0.07)	
25-30	1.09 (0.11)	
>30	1.41 (0.15)	
Pre-Pregnancy BMI Category		p = 0.6075
Underweight	1.10 (0.18)	
Healthy Weight	1.22 (0.08)	
Overweight	1.23 (0.12)	
Obese	1.04 (0.12)	
Site		**p < 0.0001*** **
Brazil-Salvador	4.10 (1.68)	
Guatemala	1.59 (0.11)	
Nicaragua	1.1 (0.13)	
Puerto Rico-North Karst	0.26 (0.024)	
Marital Status		**p = 0.0032*** **
Single	0.85 (0.10)	
Married	1.40 (0.12)	
Living Together, Not Married	1.17 (0.08)	
Locality		p = 0.46
Downtown	1.07 (0.09)	
Suburban	1.15 (0.07)	
Water Source		p = 0.0500
Town/City Water and Sewer Authority	1.08 (0.07)	
Bottled/Delivered Water	1.04 (0.08)	
Well Water	1.74 (0.33)	
Education Level		**p <.0001*** **
Less than a High School Diploma	1.44 (0.10)	
High School Diploma or GED	1.36 (0.11)	
Bachelors, Associate Degree	0.61 (0.07)	
Employment Status		p = 0.2536
Currently Employed	1.25 (0.12)	
Not Currently Employed	1.09 (0.06)	
Smoking Status		p = 0.2115
Current Smoker	2.7 (0.78)	
Former Smoker	1.02 (0.17)	
Non-Smoker	1.19 (0.06)	
Alcohol Consumption Status		p = 0.1907
Current Drinking	1.4 (0.19)	
Before Pregnancy	1.15 (0.14)	
Does Not Drink	1.08 (0.065)	
First Pregnancy		p = 0.4593
First Pregnancy	1.22 (0.10)	
Not First Pregnancy	1.13 (0.07)	
Sex of Baby		p = 0.688
Female	1.2 (1.0)	
Male	1..25 (0.07)	

*** statistically significant at the alpha = 0.05 level

Table 4.9. DEP: Crude and adjusted linear regression models

	Crude		Adjusted	
Characteristic	**Beta Coefficient (SE)**	**p-value**	**Beta Coefficient (SE)**	**p-value**
Age Category in Years				
<25	--		--	
25-30	-0.006 (0.08)	p = 0.94	0.002 (0.12)	p = 0.99
>30	0.056 (0.093)	p = 0.55	0.08 (0.11)	p = 0.81
Pre-Pregnancy BMI Category				
Underweight (≤ 18.5)	0.01 (0.12)	p = 0.93	-0.05 (0.15)	p = 0.76
Healthy Weight (18.5 < 24.9)	--		--	
Overweight (25 < 29.9)	-0.05 (0.08)	p = 0.48	-0.04 (0.10)	p = 0.70
Obese(≥ 30)	-0.2 (0.10)	**p = 0.039*** **	-0.2 (0.12)	p = 0.09
Site				
Brazil-Salvador	0.20 (0.10)	**p = 0.046*** **	-0.04 (0.14)	p = 0.77
Guatemala	0.06 (0.09)	p = 0.51	-0.013 (0.14)	p = 0.92
Nicaragua	0.10 (0.11)	p = 0.383	0.03 (0.13)	p = 0.83
Puerto Rico-North Karst	--		--	
Marital Status				
Single	--		--	
Married	-0.09 (0.10)	p = 0.36	-0.11 (0.15)	p = 0.44
Living Together, Not Married	-0.10 (0.10)	p = 0.30	-0.14 (0.13)	p = 0.28
Locality				
Downtown	-0.07 (0.08)	p = 0.33	-0.14 (0.10)	p = 0.14
Suburban	--		--	
Water Source				
Town/City Water and Sewer Authority	--		--	
Bottled/Delivered Water	-0.18 (0.08)	**p = 0.018*** **	-0.15 (0.10)	p = 0.13
Well Water	-0.03 (0.15)	p = 0.8662	-0.04 (0.16)	p = 0.82
Education Level				
Less than a High School Diploma	--		--	
High School Diploma or GED	0.07 (0.08)	p = 0.34	0.10 (0.10)	p = 0.32
Bachelors, Associate Degree	-0.01 (0.09)	p = 0.88	-0.05 (0.14)	p = 0.71
Employment Status				
Currently Employed	0.12 (0.08)	p = 0.08	0.13 (0.9)	p = 0.16
Not Currently Employed	--			
Smoking Status				
Current Smoker	0.17 (0.40)	p = 0.67	0.36 (0.46)	p = 0.44
Former Smoker	-0.04 (0.12)	p = 0.77	0.07 (0.15)	p = 0.66
Non-Smoker	--			
Alcohol Consumption Status				
Current Drinking	-0.04 (0.10)	p = 0.72	0.09 (0.13)	p = 0.70
Before Pregnancy	0.12 (0.11)	p = 0.28	0.17 (0.16)	p = 0.29
Does Not Drink	--			
First Pregnancy				
First Pregnancy	--			
Not First Pregnancy	-0.008 (0.07)	p = 0.91	0.19 (0.10)	p = 0.85
Sex of Infant				
Female	0.03 (0.08)	p = 0.73	-0.02 (0.08)	p = 0.86
Male	--			

*** statistically significant at the alpha = 0.05 level

Table 4.10. DETP: Crude and adjusted linear regression models

	Crude		Adjusted	
Characteristic	**Beta Coefficient (SE)**	**p-value**	**Beta Coefficient (SE)**	**p-value**
Age Category in Years				
<25	--		--	
25-30	-0.09 (0.06)	p = 0.1336	0.03 (0.07)	p = 0.72
>30	-0.12 (0.07)	p = 0.0642	-0.07 (0.09)	p = 0.40
Pre-Pregnancy BMI Category				
Underweight (≤ 18.5)	-0.47 (0.09)	p = 0.58	-0.0004 (0.10)	p = 0.99
Healthy Weight (18.5 < 24.9)	--		--	
Overweight (25 < 29.9)	-0.13 (0.06)	p = 0.037	-0.05 (0.07)	p = 0.495
Obese(≥ 30)	-0.34 (0.07)	**p < 0.0001*** **	-0.26 (0.08)	**p = 0.0009*** **
Site				
Brazil-Salvador	0.25 (0.07)	**p = 0.0003*** **	0.15 (1.0)	p = 0.12
Guatemala	0.36 (0.06)	**p < 0.0001*** **	**0.35 (0.09)**	**p = 0.0003*** **
Nicaragua	0.53 (0.08)	**p < 0.0001*** **	**0.37 (0.11)**	**p = 0.0006*** **
Puerto Rico-North Karst	--			
Marital Status				
Single	--		--	
Married	0.12 (0.08)	p = 0.1505	0.05 (0.10)	p = 0.49
Living Together, Not Married	0.10 (0.07)	p = 0.1505	0.02 (0.09)	p = 0.82
Locality				
Downtown	**0.11 (0.05)**	**p = 0.0388*** **	-0.038 (0.06)	p = 0.55
Suburban	--		--	
Water Source				
Town/City Water and Sewer Authority	--		--	
Bottled/Delivered Water	-0.22 (0.05)	**p < 0.0001*** **	-0.28 (0.07)	**p < 0.0001*** **
Well Water	-0.64 (0.11)	p = 0.54	-0.16 (0.12)	p = 0.18
Education Level				
Less than a High School Diploma	--		--	
High School Diploma or GED	0.01 (0.06)	p = 0.84	0.05 (0.07)	p = 0.48
Bachelors, Associate Degree	-0.25 (0.06)	**p < 0.0001*** **	-0.06 (0.09)	p = 0.48
Employment Status				
Currently Employed	0.05 (0.06)	p = 0.43	0.15 (0.07)	p =0.03
Not Currently Employed	--		--	
Smoking Status				
Current Smoker	0.6 (0.28)	p = 0.08	-0.46 (0.31)	p = 0.14
Former Smoker	0.005 (0.09)	p = 0.95	0.15 (0.10)	p = 0.11
Non-Smoker	--		--	
Alcohol Consumption Status				
Current Drinking	-0.15 (0.08)	p = 0.056	0.03 (0.09)	p = 0.74
Before Pregnancy	-0.12 (0.07)	p = 0.097	-0.12 (0.10)	p = 0.25
Does Not Drink	--		--	
First Pregnancy				
First Pregnancy	--		--	
Not First Pregnancy	-0.13 (0.05)	**p = 0.01*** **	-0.16 (0.07)	**0.017*** **
Sex of Infant				
Female	0.008 (0.05)	p = 0.88	-0.0003(0.06)	p = 0.96
Male	--		--	

*** statistically significant at the alpha = 0.05 level

Table 4.11. TCP: Crude and adjusted linear regression models

	Crude		Adjusted	
Characteristic	**Beta Coefficient (SE)**	**p-value**	**Beta Coefficient (SE)**	**p-value**
Age Category in Years				
<25	--		--	
25-30	-0.037 (0.11)	p = 0.74	-0.31 (0.12)	p = 0.09
>30	0.22 (0.13)	p = 0.09	-0.36 (0.14)	p = 0.13
Pre-Pregnancy BMI Category				
Underweight (≤ 18.5)	-0.10 (0.17)	p = 0.53	-0.11 (0.16)	p = 0.51
Healthy Weight (18.5 < 24.9)	--		--	
Overweight (25 < 29.9)	0.009 (0.118)	p = 0.08	-0.11 (0.11)	p = 0.34
Obese(≥ 30)	-0.16 (0.13)	p = 0.24	0.05 (0.13)	p = 0.69
Site				
Brazil-Salvador	2.73 (0.11)	**p < 0.0001*****	2.8 (0.17)	**p < 0.0001*****
Guatemala	1.79 (0.10)	**p < 0.0001*****	1.8 (0.16)	**p < 0.0001*****
Nicaragua	1.42 (0.13)	**p < 0.0001*****	1.55 (0.18)	**p < 0.0001*****
Puerto Rico-North Karst	--		--	
Marital Status				
Single	--		--	
Married	0.5 (0.15)	**p = 0.01*****	-0.13 (0.14)	p = 0.35
Living Together, Not Married	0.32 (0.13)	**p = 0.007*****	0.03 (0.16)	p = 0.86
Locality				
Downtown	-0.07 (0.11)	p = 0.46	-0.08 (0.10)	p = 0.49
Suburban	--		--	
Water Source				
Town/City Water and Sewer Authority	--		--	
Bottled/Delivered Water	-0.042	p = 0.69	0.11 (0.2)	p = 0.57
Well Water	0.48 (0.11)	**p =0.02*****	0.20 (0.11)	p = 0.07
Education Level				
Less than a High School Diploma	--		--	
High School Diploma or GED	-0.69 (0.11)	p = 0.53	0.14 (0.11)	p = 0.23
Bachelors, Associate Degree	-0.85 (0.12)	**p < 0.0001*****	0.126 (0.15)	p = 0.42
Employment Status				
Currently Employed	0.13 (0.12)	p = 0.25	0.13 (0.12)	p = 0.27
Not Currently Employed	--		--	
Smoking Status				
Current Smoker	0.82 (0.54)	p = 0.134	0.33 (0.52)	p = 0.52
Former Smoker	-0.156 (0.17)	p = 0.377	-0.05 (0.18)	p = 0.75
Non-Smoker	--		--	
Alcohol Consumption Status				
Current Drinking	0.06 (0.13)	p = 0.67	0.06 (0.15)	p = 0.51
Before Pregnancy	0.28 (0.16)	p = 0.069	0.12 (0.18)	p = 0.49
Does Not Drink	--			
First Pregnancy				
First Pregnancy	--		--	
Not First Pregnancy	-0.07 (0.10)	p = 0.45	0.22 (0.11)	p = 0.30
Sex of Infant				
Female	0.04 (0.10)	p = 0.68	0.09 (0.09)	p = 0.33
Male	--		--	

*** statistically significant at the alpha = 0.05 level

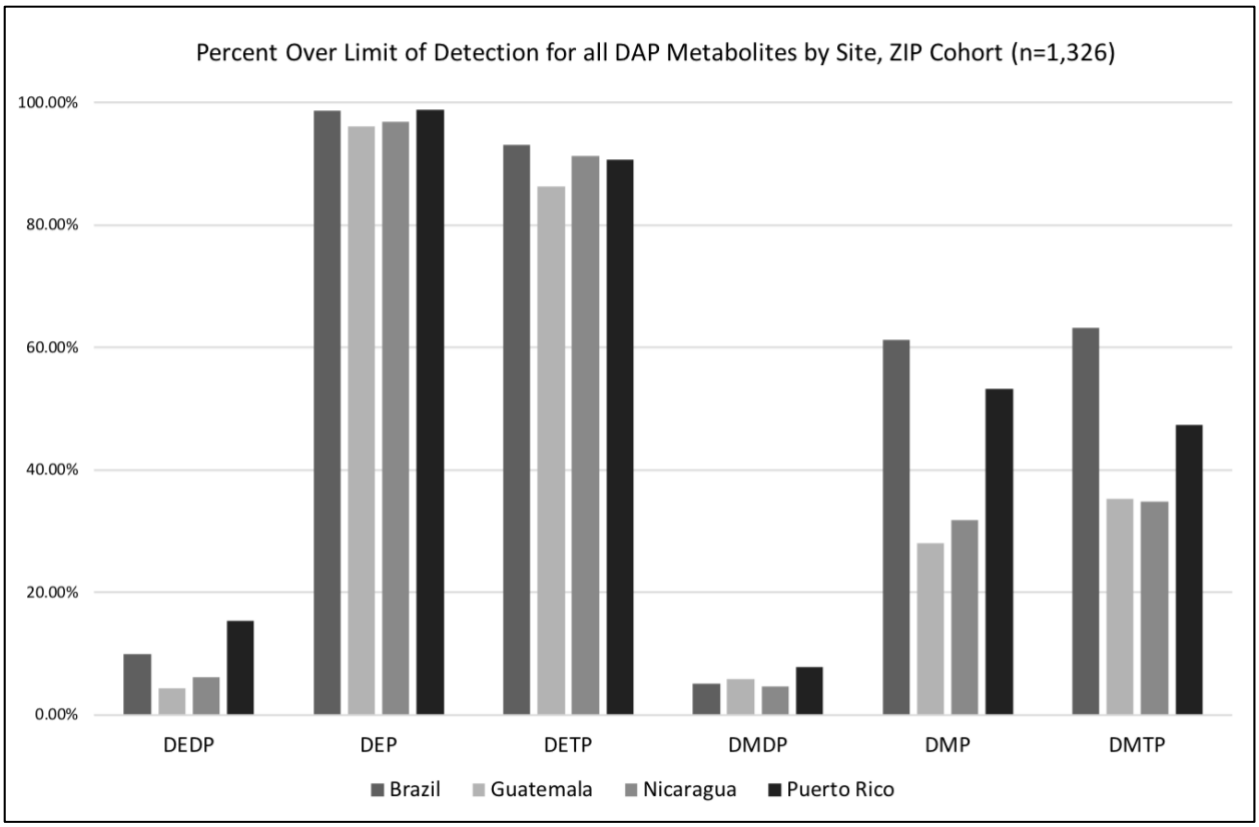


Figure 4.1 Percent over Limit of Detection for all Organophosphate Metabolites by Site

Diethyldithiophosphate (DEDP), Diethylphosphate (DEP), Diethylthiophosphate (DETP), Dimethyldithiophosphate (DMDP), Dimethylphosphate (DMP), Dimethylthiophosphate (DMTP), LOD for all metabolites = 0.5 ng/mL

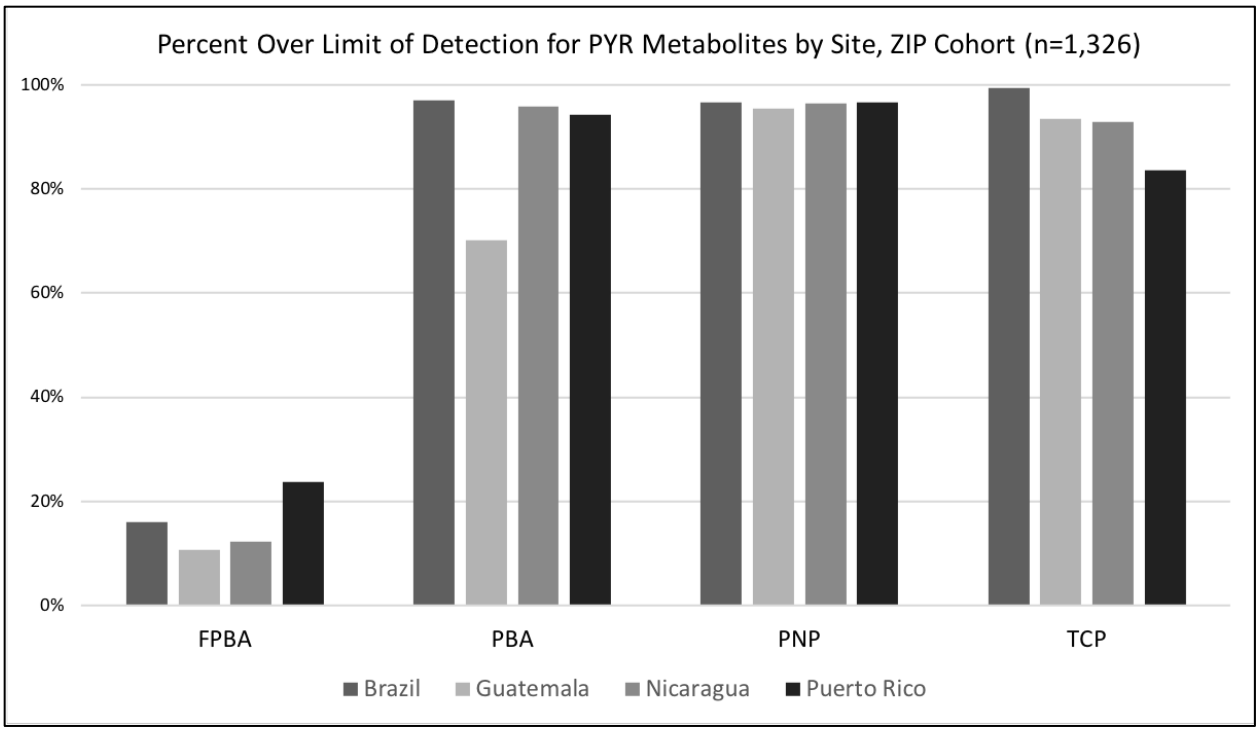


Figure 4.2. Percent over Limit of Detection for all Pyrethroid Metabolites by Site

3-phenoxybenzoic acid (PBA), 4-fluoro-3-phenoxybenzoic acid (FPBA), 4-nitrophenol (PNP), and 3,5,6-trichloro-2- pyridinol (TCP)

CHAPTER 5

CHANGES IN ORGANOPHOSPHATE AND PYRETHROID METABOLITE LEVELS ACROSS GESTATION AMONG THE ZIKA AND INFANTS IN PREGNANCY COHORT

[1]Mallis, N., Welton, M.., Knight, J., Shen, Y., Glenn, T., Cordero, JF

To be submitted to Environment International

Abstract

Background: Organophosphates (OPs) and pyrethroids (PYRs) are insecticides used worldwide in large scale farming, home applications, and insect repellents. Studies suggest that OP and PYR exposure during pregnancy are associated poor birth outcomes, but few studies have investigated how OP and PYR urine metabolite levels change across gestation.

Objective: Examine changes in organophosphate and pyrethroid urine biomarker levels over time during pregnancy using data from the Zika in Infants and Pregnancy (ZIP) cohort.

Methods: 1,326 pregnant persons were recruited from ZIP research sites in Puerto Rico (North Karst), Nicaragua (Managua), Brazil (Salvador), and Guatemala (Guatemala City). Participants were first interviewed about demographic characteristics and followed throughout pregnancy until delivery. Urine samples were collected at regular visits and analyzed for pesticide biomarker concentrations by Mount Sinai's Children's Health Exposure Analysis Resource (CHEAR). Six OP biomarkers were measured: Diethyldithiophosphate (DEDP), Diethylphosphate (DEP), Diethylthiophosphate (DETP), Dimethyldithiophosphate (DMDP), Dimethylphosphate (DMP), Dimethylthiophosphate (DMTP), 3,5,6-trichloro-2- pyridinol (TCP), and three PYR biomarkers: 3-phenoxybenzoic acid (PBA), 4-fluoro-3-phenoxybenzoic acid (FPBA), 4-nitrophenol (PNP). We present spline plots and stratified box plots for each metabolite to visualize relationships with gestational age in weeks. We ran univariate and adjusted longitudinal models with gestational age in weeks as the predictor and the log of each metabolite as the outcome. We also ran these models with quadratic terms to explore non-linear relationships.

Results: TCP displayed evidence of increasing levels throughout gestation in both the spline plots and the stratified boxplots while other metabolites did not reveal patterns. The longitudinal model with log TCP as the outcome showed a significant linear relationship with gestational age [TCP Beta(SE) = 0.058(0.008) $p<0.0001$]. Additionally, longitudinal modeling revealed a significant linear relationship with gestational age and log PNP [Beta(SE) = 0.02(0.001), $p<0.0001$].

Conclusion/Discussion: Among the five metabolites examined in this study, TCP (a biomarker for chlorpyrifos) was the only analyte that appeared to be increasing throughout gestation. Chlorpyrifos is used in outdoor pest control to kill mosquitos. Pregnant persons might have increased use during pregnancy to protect from vector borne diseases. Changes in TCP levels across gestation could reflect exposure due to seasonality (higher spraying in certain times of the year which could lead to higher exposure level). Future studies should examine time of year and exposure levels. Data from this study was prepared by our team to be publicly available through CHEAR. These results will aid future analyses with this data source and add to growing knowledge on pesticide exposure during pregnancy.

Introduction

Organophosphates (OPs) and pyrethroids (PYRs) are both insecticides widely used across the world. Organophosphates are mostly applied in large scale farming, while pyrethroids are used in large scale farming, home applications, and in insect repellents that are sprayed directly on clothes and mosquito nets (2). OPs and PYRs came into prominence in the 1970's and were thought to be a safer option for pest control compared to organochlorines (DDT), which were banned in the US due to the deleterious effects on wildlife, persistence in the environment, and association with adverse health outcomes in humans (1). Common organophosphates pesticides include parathion, diazinon, malathion, and chlorpyrifos (2). Organophosphates poison insects and other pests by targeting acetylcholinesterase enzymes found in nerve endings, which causes organs to be overstimulated by excess acetylcholine and ultimately kills the insect via neurotoxicity (1). Permethrin, resmethrin, and sumithrin are common pyrethroids that are used around the world (2). Pyrethroids target the voltage-gated sodium channels in the nervous system in insects, causing paralysis and death (3). Both organophosphates and pyrethroids have been instrumental in advancing agriculture and public health efforts to combat vector borne diseases, but acute and long-term exposures have been associated with negative effects in humans (1).

Humans can be exposed to small amounts of OPs and PYRs through food and water intake, inhalation, and direct contact (1). Individuals who work in agriculture and other occupations that regularly use pesticides are at a higher risk of exposure (4). Similar to their intended effect on pests, OPs bring about toxic effects on humans by binding to the acetylcholinesterase enzyme and result in high levels of acetylcholine to build up. Both acute and long-term exposure can cause problems in the respiratory, cardiovascular, and nervous system (2). Several studies examining organophosphate urine biomarkers have found that OP exposure

during pregnancy was associated with low birth weight, smaller head circumference, and smaller birth length (7-9). Existing literature also suggests that OP exposure during pregnancy is associated with neurodevelopmental and behavioral issues (10, 11). With pyrethroids, acute poisoning in humans can cause coughing and bronchospasm, nausea and vomiting (1). Studies have also suggested that pyrethroids have adverse effects on male sperm concentration and motility (19, 20). A recent study published in 2022 in found that first and second trimester exposures to high levels of PYRs were associated with poorer infant neurodevelopmental outcomes by age one (12).

Measuring urine biomarker levels during pregnancy provides an indication of pesticide exposure, but it is important to note that these measurements reflect recent exposures that would have occurred within a few days of the sample collection (43). This poses a design challenge when using urine biomarkers for studies that examine organophosphate and pyrethroid exposures and pregnancy outcomes. The present analysis will use data from the Zika in Infants and Pregnancy (ZIP) Cohort to examine how urine metabolite levels (DEP, DETP, PNP, PBA, and TCP) change across gestation.

Methods

Data used for this analysis was collected as part of the Zika in Infants and Pregnancy (ZIP) study. Details on the ZIP cohort are outlined previously in chapter 1 (62). Briefly, for our sub study, 1,326 pregnant persons were recruited from ZIP research teams in Puerto Rico (North Karst), Nicaragua (Managua), Brazil (Salvador), and Guatemala (Guatemala City). Women 15 years old or older who planned to give birth in the study regions and were not participating in other research studies were deemed eligible. Participants were first interviewed in an initial visit

for baseline demographics, medical history, and current pregnancy information. Participants then had monthly targeted clinical examinations where they were interviewed about occupational and household characteristics and other health behaviors. Pregnant persons enrolled in the study were followed through delivery and birth outcomes were recorded.

Biologic samples were collected at scheduled visits including blood and urine, used for Zika virus (ZIKV) analysis and specimen repositories. Additional urine samples were collected at visits for the pesticide analysis. Each participating site collected the urine samples and stored in -20-degree freezers in 15-50 ml conical tubes. Once the urine sample collection was completed, the samples were transported to the University of Georgia and stored in -20-degree freezers in 15-50 ml conical tubes. Samples were aliquoted into 5 ml vials before shipment to Mount Sinai's Children's Health Exposure Analysis Resource (CHEAR) for pesticide analysis.

CHEAR Pesticide Analysis

2,705 urine samples were submitted to CHEAR and tested for organophosphate and pyrethroid pesticides. Six biomarkers, Diethyldithiophosphate (DEDP), Diethylphosphate (DEP), Diethylthiophosphate (DETP), Dimethyldithiophosphate (DMDP), Dimethylphosphate (DMP), and Dimethylthiophosphate (DMTP) were tested to detect organophosphate levels. Four biomarkers, 3-phenoxybenzoic acid (PBA), 4-fluoro-3-phenoxybenzoic acid (FPBA), 4-nitrophenol (PNP), and 3,5,6-trichloro-2- pyridinol (TCP) were tested to detect pyrethroid levels. The Limit of Detection (LOD) values reported were the average of the daily LODs for the whole study. The daily LODs were used to identify the valid values for each sample. Quality control pairs were used to measure of precision based on the percent of the difference between duplicate sample concentrations relative to the mean of the duplicate sample concentrations per subject.

We merged the lab data from the CHEAR urine analysis with epidemiologic data from the ZIP study.

Exposure Ascertainment

Biomarkers with a limit of detection (LOD) greater than 70% were included in this analysis. For all organophosphate biomarkers, the LOD was 0.5 ng/mL. For all pyrethroid biomarkers, the LOD was 0.10 ng/mL. Out of the seven OP biomarkers measured, DEP (98% detect) DETP (90% detect), and TCP (96% detect), had LODs greater than 70% and were used as primary exposures in the analysis. DEDP (8% detect), DMDP (6% detect), DMP (56% detect), DMTP (49% detect), and FPBA (17% detect) were excluded from the analysis. Both PYR biomarkers measured, PBA (90% detect) and PNP (96% detect), had LODs greater than 70% and were used as the primary exposures in this analysis. Among biomarkers that were included, samples with a concentration below the LOD were imputed using a standard formula in which the LOD is divided by the square root of two (54). Then, for each biomarker, concentration levels were divided by the creatinine level to correct for urine dilution, [creatinine corrected level = metabolite level concentration / creatinine concentration] (53). Gestational age in weeks at the time of collection was calculated for each urine sample used in the study based on reported LMP. For this analysis, we used all available samples from each participant (n=1,362). Six hundred and sixty-seven participants had only one sample collection and 659 had two or more. Collections were spread across gestation with most occurring in the second (32%) and third trimesters (45.5%).

Statistical Analysis

To display time trends visually, we plotted splines, which use a smoothing function to show the average trajectory of metabolite concentrations by gestational age in weeks. We present spline plots for DEP, DETP, TCP, PNP, and PBA. We also used ad hoc methods and stratified the splines by maternal characteristic levels in an attempt to pinpoint patterns. To further visualize possible relationships across time, we plotted boxplots by week of gestation for each biomarker, which helped visualize if certain metabolite levels tended to be higher at different points in pregnancy.

For modeling, we ran crude and adjusted longitudinal models for each biomarker to quantify linear trends in metabolite levels across gestation, accounting for the correlation of repeated measurements. We also ran crude and adjusted models with a quadratic term to examine possible non-linear relationships with gestational age and metabolite levels. For all models, we used the log of the creatinine corrected biomarker. Models were adjusted for characteristics found to be significantly associated with the metabolites in aim 1. For DEP, characteristics included site and primary water source. For DETP, it included site, primary water source, BMI category, education level, and first pregnancy or not. For PNP, age category, BMI category, site, marital status, locality, water source, education level and employment status were included. For PBA, characteristics include site, BMI category, marital status, locality, water source, education level, smoking, and alcohol consumption and for TCP, Included site, marital status and education.

Results

Study population

Characteristics of the study main population (n=1,327) have been described in Aim 1. Briefly, the majority of the participants were under 25 years of age (54.22%), in the healthy BMI category (45.32%), and came from the Guatemala study site (37.03%) (Table 1). Most of the participants were either married or living with a partner (84%) and currently unemployed (69%). Forty-five percent of the participants did not have a high school diploma. The distribution of characteristics was similar for the group of 659 with multiple sample collections, but there were variations by site (Table 1). A majority of the participants with multiple samples resided in Puerto Rico (42.03%) and Brazil (40.21%). While participants from Guatemala were a majority in the full study population, they only made-up 10.6 percent of the group with multiple samples collected.

Organophosphates

The spline plots for DEP and DETP metabolite levels did not reveal evidence of on average increases throughout gestation (Figures 5.1-5.2), although some individual trajectories seemed to follow a quadratic relationship with higher concentrations in the middle of gestation and lower concentrations later. Based on the plots, on average, levels stayed the same throughout pregnancy for both DEP and DETP. For TCP, levels appear to be steadily increasing throughout gestation (Figure 5.5). In the ad hoc analysis, we clustered data based on subgroups of maternal characteristics (site, employment, and water source) and plotted the splines by level of these variables, but no visual differences were apparent in these plots (Figures 5.11-5.14) Both the

crude and the adjusted models with log DEP as the outcome did not show a significant linear or quadratic relationship (Table 5.2). For log DETP, the longitudinal model showed a significant linear relationship with gestational age [Beta(SE) = 0.005(0.001), p=0.008] , but this beta coefficient was small in magnitude, so it might not be clinically significant (Table 5.3). Both the crude and adjusted models with log DETP as the outcome show a significant quadratic relationship across gestation, but the coefficient was small in magnitude Table 5.3). For TCP, the means appear to be increasing across gestation (Figure 5.10). For TCP, the longitudinal model showed significant linear relationships with gestational age in both the crude and adjusted model [TCP Beta(SE) = 0.058(0.008) p<0.0001] (Table 5.6).

Pyrethroids

The spline plots for PNP and PBA metabolite levels did not reveal evidence of increases throughout gestation (Figures 5.3-5.4). Based on the plots, on average, PNP and PBA levels stayed the same throughout pregnancy. In the ad hoc analysis, we clustered data based on subgroups of maternal characteristics (site, employment, and water source) and plotted the splines by level of these variables, but no visual differences were apparent in these plots (Figures 5.15-5.20). In the boxplots by gestational age in weeks for PNP and PBA, the distribution across gestation does not seem to be changing (figures 5.8-5.9). For both PNP the longitudinal model showed significant linear relationships with gestational age in both the crude and adjusted model [PNP Beta(SE) = 0.02(0.001), p<0.0001], (Table 5.4).

Discussion

This analysis examined how urine metabolite levels for organophosphates and pyrethroids changed across gestation among participants enrolled in a diverse pregnancy cohort. The spline plots revealed evidence that TCP metabolite levels were increasing throughout gestation in our study, while the splines for other metabolites did not appear to be changing throughout gestation. The longitudinal model with TCP as the outcome revealed a significant linear relationship with gestational age [TCP Beta(SE) = 0.02 $p<0.0001$] and the longitudinal model with DETP as the outcome revealed a significant linear relationship with gestational age [DETP Beta(SE) = 0.005(0.001), $p=0.008$]. Additionally, longitudinal modeling revealed a significant linear relationship with gestational age and log PNP [Beta(SE) = 0.02(0.001), $p<0.0001$]. Among the five metabolites examined in this study, TCP was the only one that appeared to be increasing throughout gestation.

Few studies have examined time trends across gestation with regard to organophosphate and pyrethroids biomarkers, with some reporting increases across, some reporting decreases, and some reporting no change. One study conducted in the Netherlands did not see a change in DAP metabolite levels across gestation and reported similar median DAP metabolite levels in each trimester, which is consistent with the findings in our study (50). Another study specifically examined pyrethroid biomarkers and reported that PBA levels remined the same during pregnancy, but other pyrethroid biomarkers slightly increased (12). One study found sharp increases in organophosphate metabolites just after birth, but mean levels were similar during gestation (44). Another cohort study that examined endocrine disrupting chemicals (EDC) in

urine across gestation and reported that persistent EDCs tended to decrease across pregnancy time points (52).

TCP is metabolite for chlorpyrifos, an organophosphate used in agriculture crops and structured pest control to target mosquitos. TCP is also a metabolite for triclopyr, an herbicide used in home and lawn gardening. Changes in TCP levels across gestation also reflect exposure due to seasonality (higher spraying in certain times of the year which could lead to higher exposure levels). Further, the results of this study should be interpreted within the context of the zika outbreak in the Americas during 2015-2016. Infants of pregnant persons infected with zika can be born with microcephaly, other serious birth defects and may have serious disabilities. Participants in our study resided in Brazil, Guatemala, Nicaragua, and Puerto Rico, all places in which zika was a public health issue at the time of data collection (60). During urine specimen collection, participants might have used insecticides in their homes, yards, on their clothing, and in mosquito nets more frequently than previous years

There are some limitations to consider when interpreting the results. While the study team intended to collect one urine sample for each trimester, data collection was not as uniform as planned. Only 166 (12.2%) had a sample taken from each trimester, although we did obtain multiple (2 or 3) samples from 659 (49.9%) of the participants in our study. Some participants had multiple samples taken at different trimesters, but none during other trimesters. Further, data was not available on certain covariates that would have been of interest. Information on dietary (fruit and vegetable) intake, type of occupation (agriculture vs other types of employment), and pesticide usage habits would have been helpful in the analysis and interpretation of the longitudinal results.

These results will aid future analyses with this data source and add to growing knowledge on pesticide exposure during pregnancy. In our analysis, TCP (an organophosphate) seemed to increase with gestational age while other metabolites remained stable. Future research with this dataset and other cohorts should examine this relationship and take into account seasonality to identify higher months of exposure. Further, studies should collect data on direct usage of organophosphates and pyrethroids in populations who work directly with pesticides in agriculture or gardening to examine how exposure profiles change over time.

Table 5.1. Maternal Characteristics of Population, Zika in Infants and Pregnancy Cohort (ZIP)

Characteristic	**Total (n=1,326)**	**Group with more than one sample collection (n = 659)**
Age Category in Years, N(%)		
<25	719 (54.22)	326 (49.4)
25-30	366 (27.6)	189 (28.26)
>30	238 (17.95)	142 (21.6)
Missing	3 (0.23)	2 (0.3)
Pre-Pregnancy BMI Category (Kilograms/Height^2)		
Underweight ($\leq$ 18.5)	133 (10.03)	71 (10.77)
Healthy Weight (18.5 < 24.9)	601 (45.32)	275 (41.73)
Overweight (25 < 29.9)	338 (25.49)	181 (27.47)
Obese($\geq$ 30)	237 (17.87)	123 (18.66)
Missing	17 (1.28)	9 (1.37)
Site		
Brazil-Salvador	294 (22.17)	265 (40.21)
Guatemala	491 (37.03)	70 (10.62)
Nicaragua	195 (14.7)	47 (7.13)
Puerto Rico-North Karst	346 (26.09)	277 (42.03)
Marital Status		
Single	211 (15.91)	152 (23.07)
Married	404 (30.47)	143 (21.7)
Living Together, Not Married	704 (53.09)	360 (54.63)
Missing	7 (.053)	4 (0.61)
Education Level		
Less than a High School Diploma	609 (45.9)	200 (30.35)
High School Diploma or Equivalent	416 (31.37)	228 (34.6)
Associate Degree, Bachelor's Degree or Higher	296 (22.32)	227 (34.45)
Missing	5 (0.38)	4 (0.61)
Employment Status		
Currently Employed	307 (23.15)	203 (30.8)
Not Currently Employed	923(69.61)	411 (62.37)
Missing	96 (6.9)	45 (6.83)
Smoking Status		
Current Smoker	10 (0.75)	8 (1.21)
Former Smoker	111 (8.37)	63 (9.56)
Never Smoked	1,045 (78.81)	491 (74.51)
Missing	160 (12.07)	97 (14.72)
First Pregnancy		
First Pregnancy	501 (37.78)	273 (41.43)
Not First Pregnancy	820 (61.84)	382 (57.97)
Missing	5 (0.38)	4 (0.61)
Sex of Infant		
Female	553 (41.7)	295 (44.76)
Male	602 (45.3)	322 (48.6)
Missing	171 (12.9)	42 (6.37)

Table 5.2 Longitudinal models with gestational age in weeks as predictor and log creatinine corrected DEP as outcome

	Crude		Adjusted[a]	
	Coefficient (SE)	**p-value**	**Coefficient (SE)**	**p-value**
Linear Model				
Gestational Age (wks)	0.003 (0.002)	p = 0.13	0.002 (0.002)	p = 0.30
Quadratic Model				
Gestational Age	0.02 (0.01)	p = 0.04	0.02 (0.01)	p = 0.05
Gestational Age*Gestational Age	-0.0004 (0.0002)	p = 0.08	-0.0004 (0.0002)	p = 0.08

[a]DEP models adjusted for site and primary water source

Table 5.3 Longitudinal models with gestational age in weeks as predictor and log creatinine corrected DETP as outcome

	Crude		Adjusted[a]	
	Coefficient (SE)	**p-value**	**Coefficient (SE)**	**p-value**
Linear Model				
Gestational Age (wks)	0.006 (0.001)	p = 0.008*	0.002 (0.002)	p = 0.40
Quadratic Model				
Gestational Age	0.05 (0.009)	p < 0.001*	0.03 (0.01)	p = 0.004*
Gestational Age*Gestational Age	-0.0009(0.0002)	p < 0.001*	-0.0006 (0.0002)	p = 0.006*

[a]DETP models adjusted for site, primary water source, locality, education level, and pre-pregnancy BMI

Table 5.4. Longitudinal models with gestational age in weeks as predictor and PNP as outcome

	Crude		Adjusted[a]	
	Coefficient (SE)	**p-value**	**Coefficient (SE)**	**p-value**
Linear Model				
Gestational Age (wks)	0.02 (0.001)	p<.0001*	0.009 (0.001)	p<.0001*
Quadratic Model				
Gestational Age	0.02 (0.007)	p = 0.003	0.004 (0.007)	p =0.58
Gestational Age*Gestational Age	-0.0001 (0.0001)	p = 0.38	0.0001 (0001)	p =0.51

[a]adjusted for site, age category, BMI category, site, marital status, locality, water source, education level and employment status

Table 5.5. Longitudinal models with gestational age in weeks as predictor and PBA as outcome

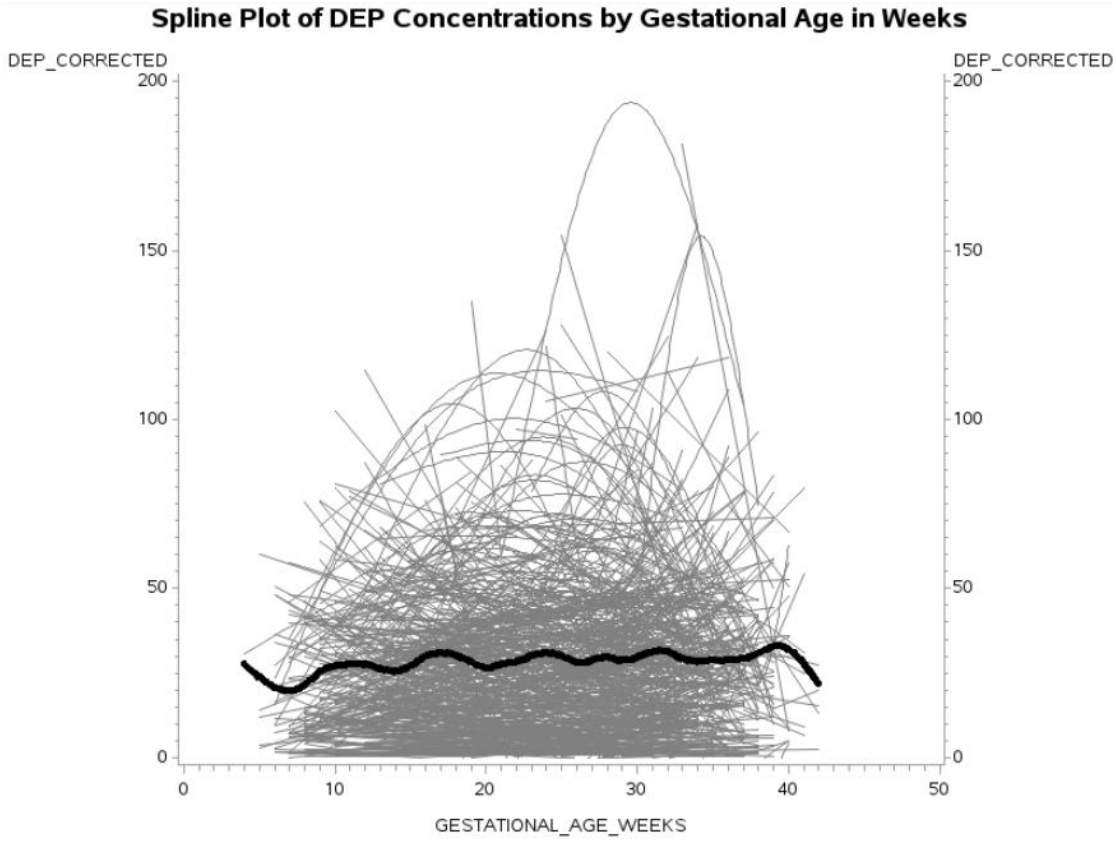
	Crude		Adjusted[a]	
	Coefficient (SE)	**p-value**	**Coefficient (SE)**	**p-value**
Linear Model				
Gestational Age (wks)	-0.0082(0.002)	p = 0.73	0.003 (0.002)	p = 0.21
Quadratic Model				
Gestational Age	-0.0008 (0.01)	p = 0.46	0.016 (0.01)	p = 0.19
Gestational Age*Gestational Age	0.0001(0.0002)	P = 0.49	-0.0003 (0.0002)	p = 0.28

[a]adjusted for site, BMI category, marital status, locality, water source, education level, smoking, and alcohol consumption

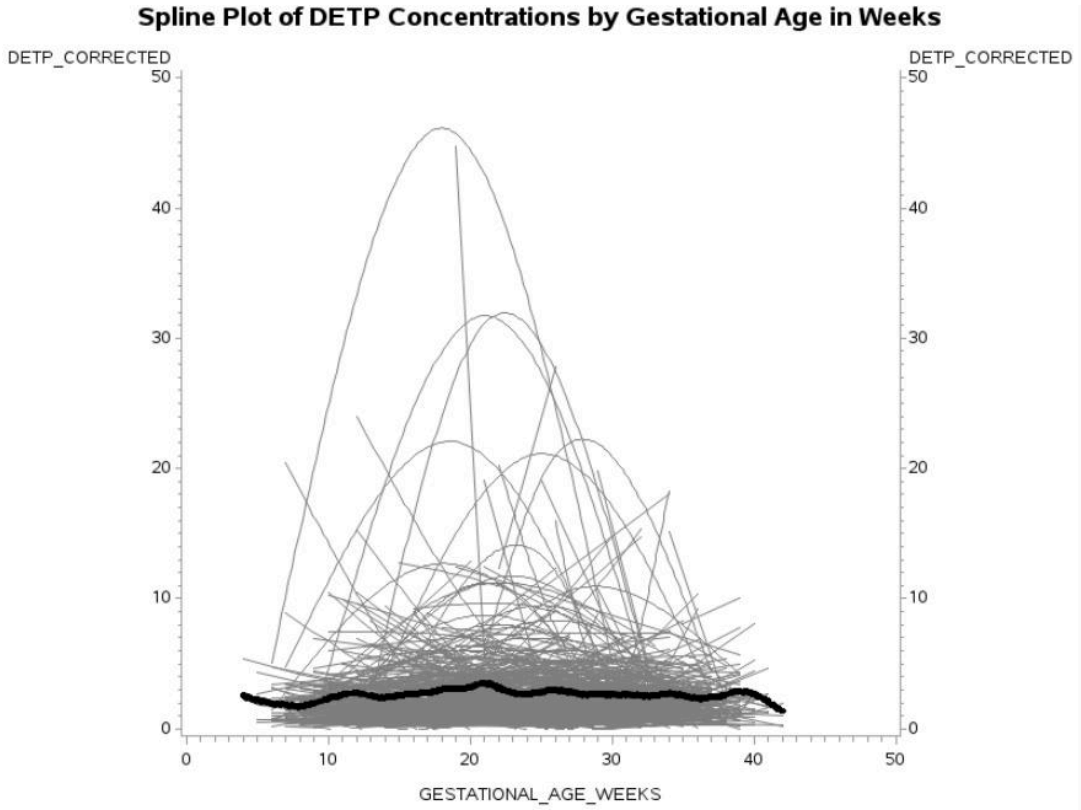
Table 5.6. Longitudinal models with gestational age in weeks as predictor and TCP as outcome

	Crude		**Adjusted**[a]	
	Coefficient (SE)	**p-value**	**Coefficient (SE)**	**p-value**
Linear Model				
Gestational Age (wks)	0.058(0.008)	p < 0.0001*	0.004 (0.003)	p = 0.11
Quadratic Model				
Gestational Age	0.049 (0.016)	p = 0.0015	0.003 (0.015)	p = 0.84
Gestational Age*Gestational Age	-0.0005 (0.0003)	p = 0.1136	0.00003 (0.0003)	p = 0.9

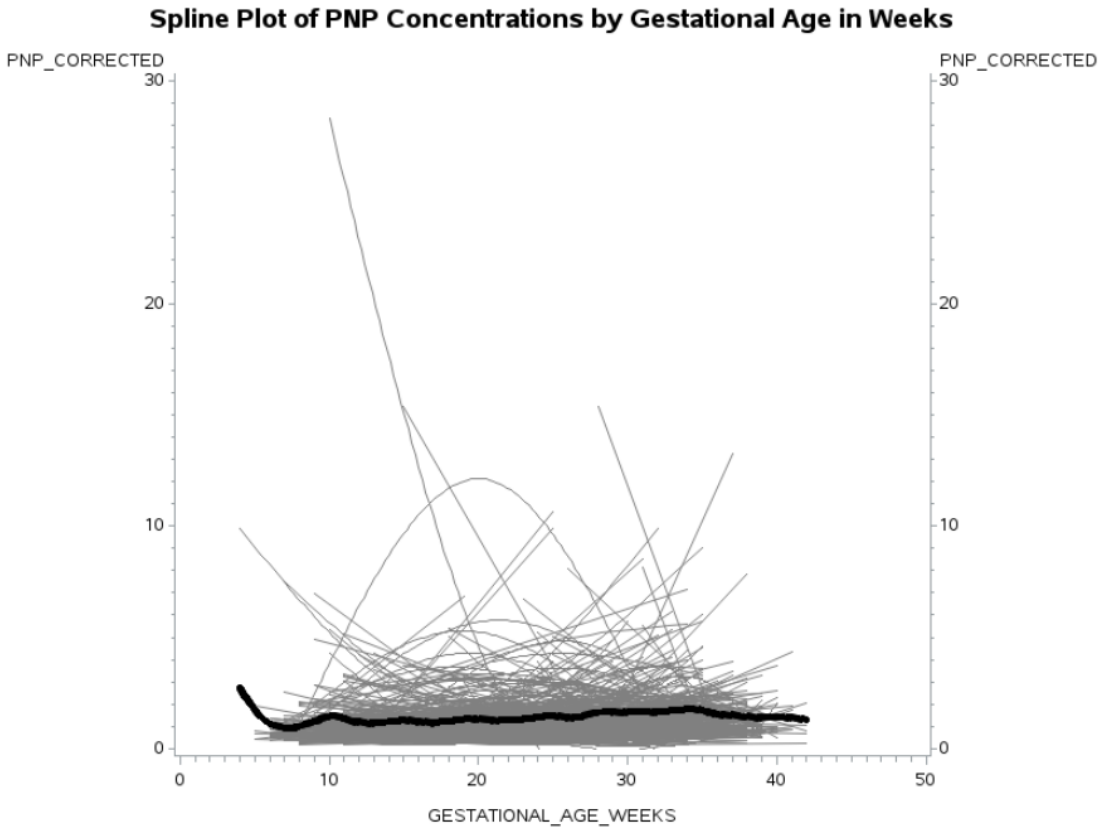
[a]adjusted for site, marital status and education



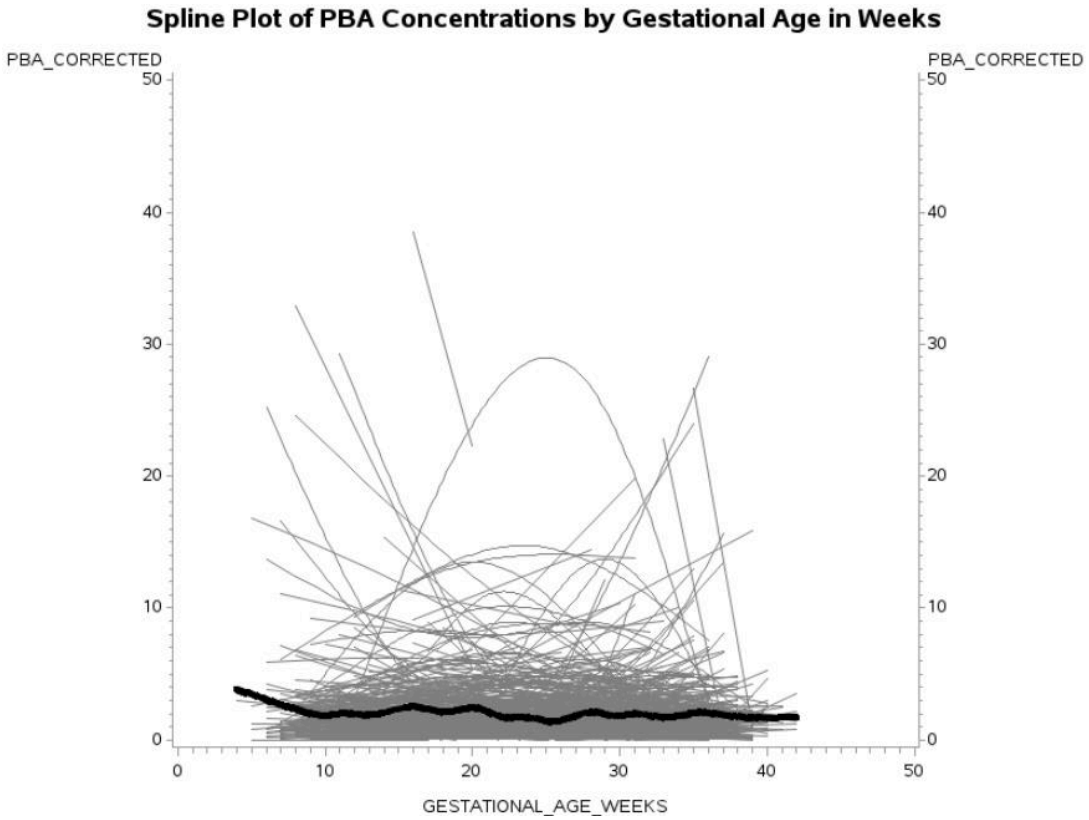
5.1. Spline Plot of DEP Concentrations by Gestational Age, ZIP Cohort (n=1,326)



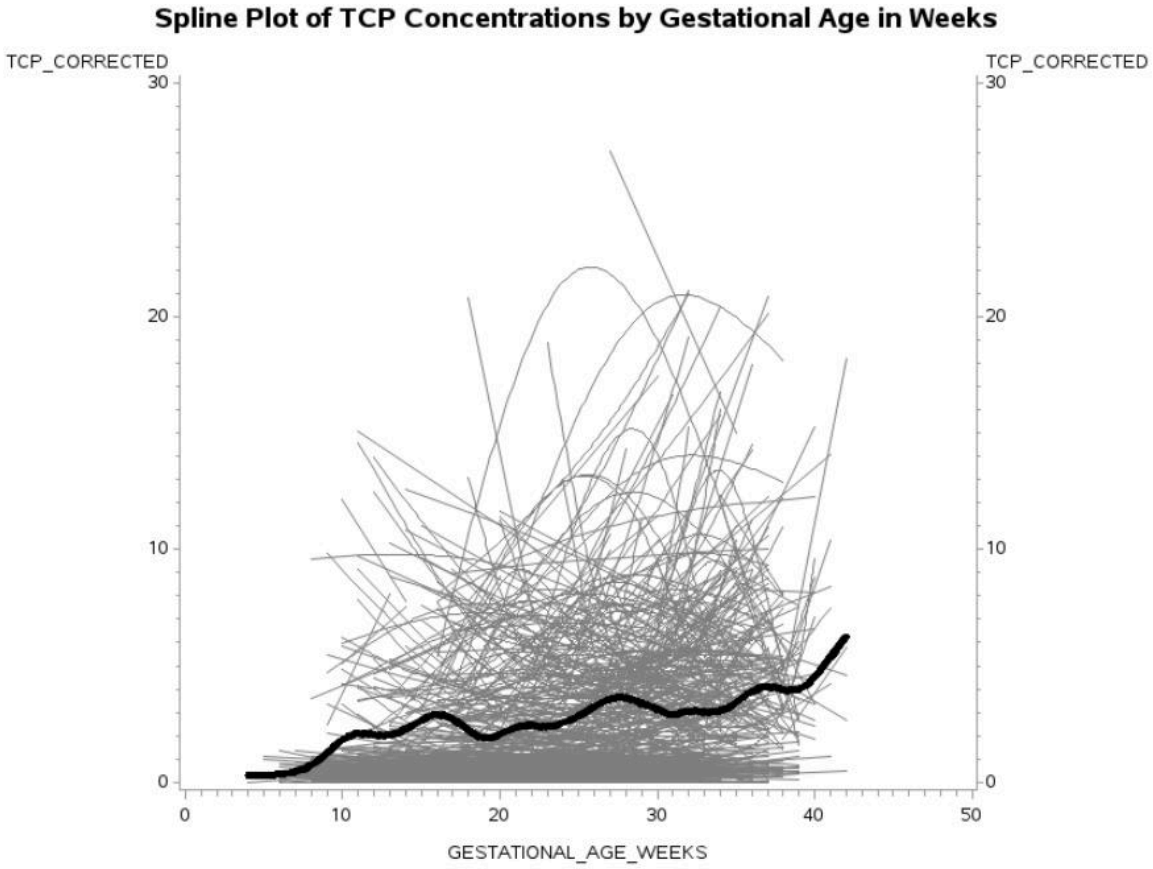
5.2. Spline Plot of DETP Concentrations by Gestational Age, ZIP Cohort (n=1,326)



5.3. Spline Plot of PNP Concentrations by Gestational Age, ZIP Cohort (n=1,326)



5.4. Spline Plot of PNP Concentrations by Gestational Age, ZIP Cohort (n=1,326)



5.5. Spline Plot of TCP Concentrations by Gestational Age, ZIP Cohort (n=1,326)

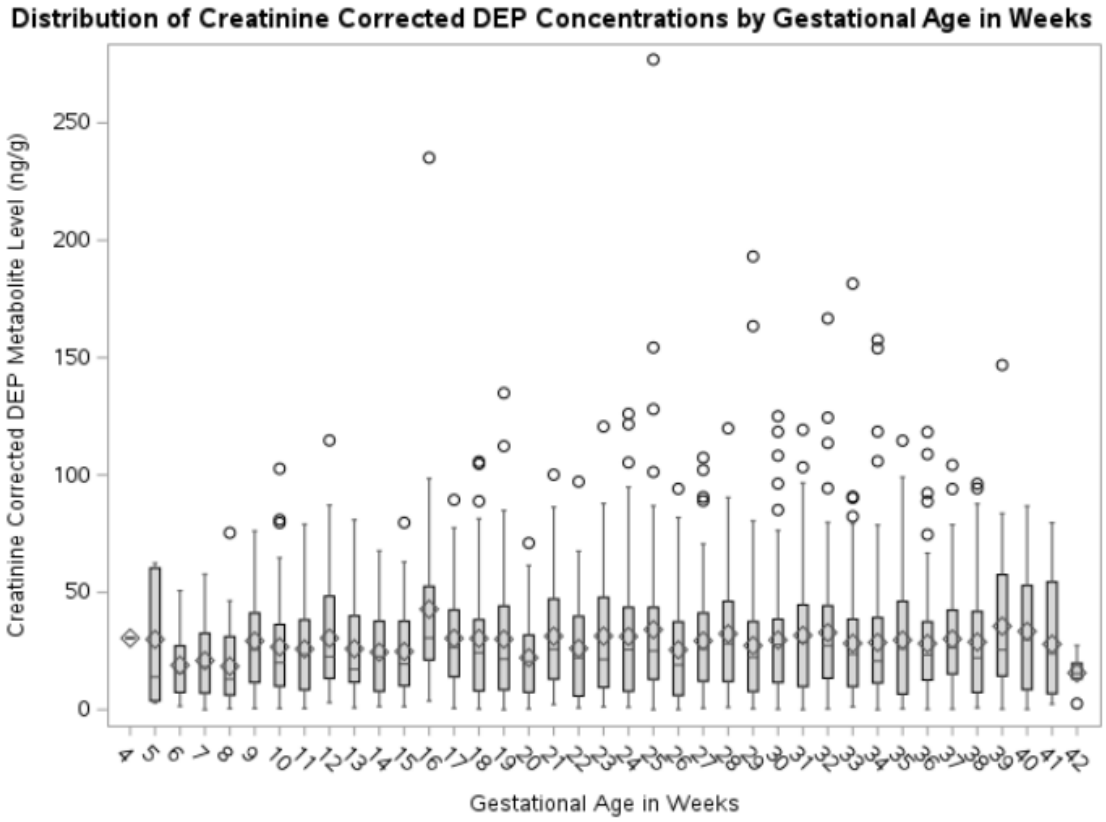


Figure 5.6. Distribution of DEP Concentrations by Gestational Age (n=1,362)

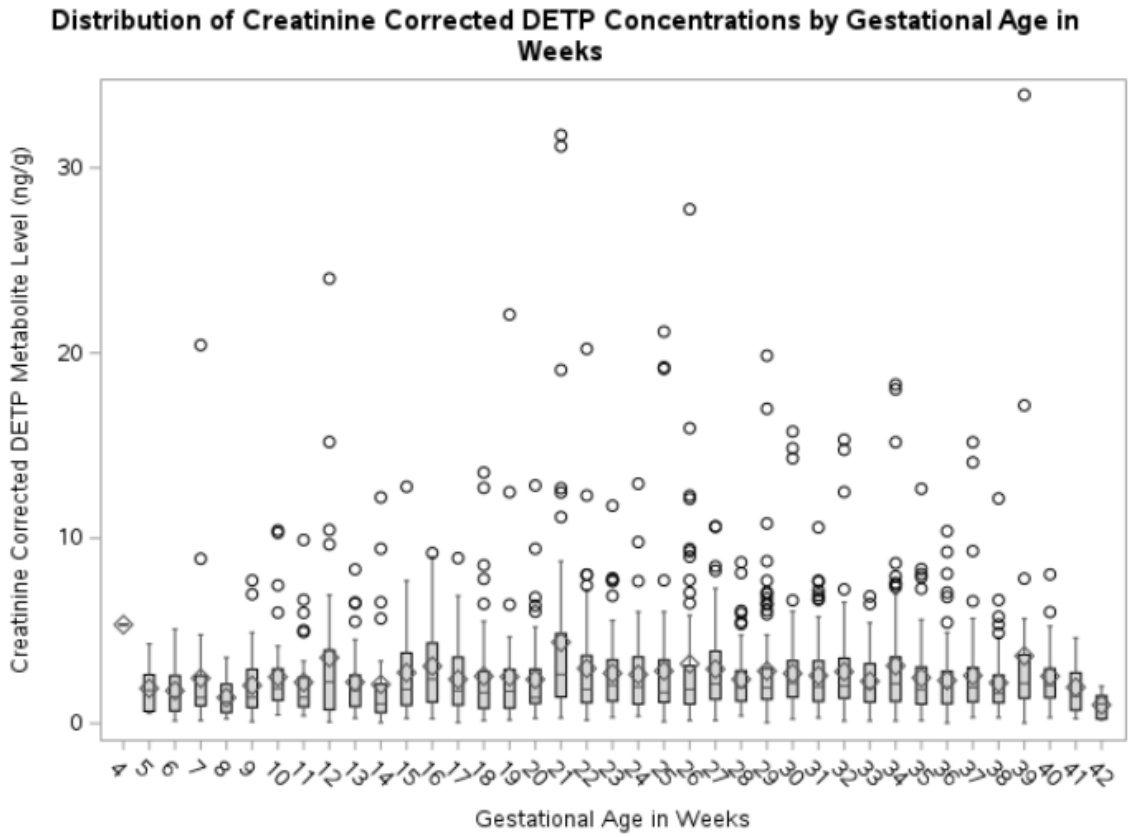


Figure 5.7. Distribution of DETP Concentrations by Gestational Age (n=1,362)

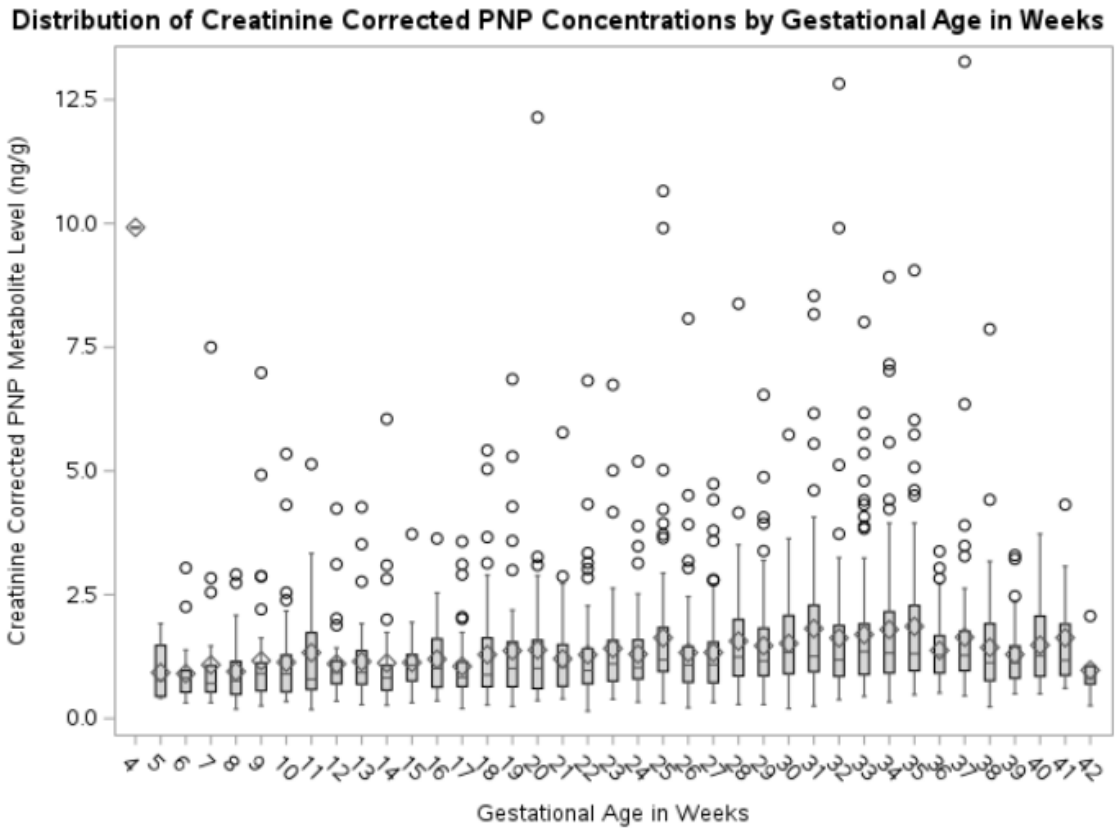


Figure 5.8. Distribution of PNP Concentrations by Gestational Age (n=1,362)

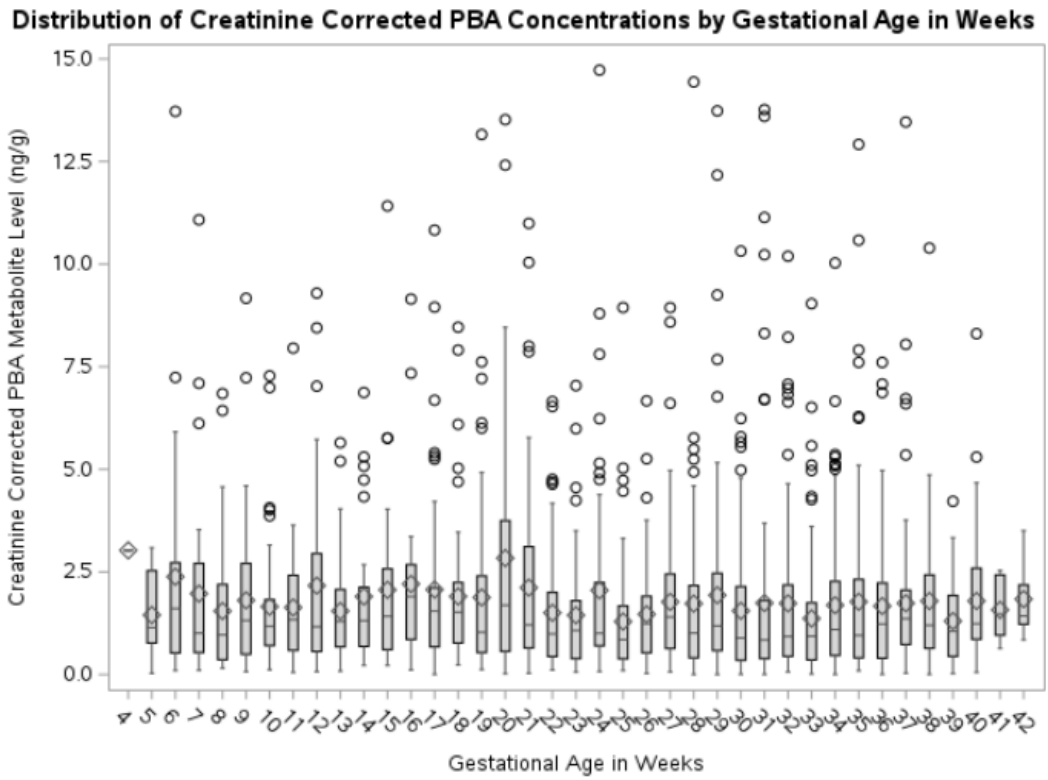


Figure 5.9. Distribution of PBA Concentrations by Gestational Age (n=1,362)

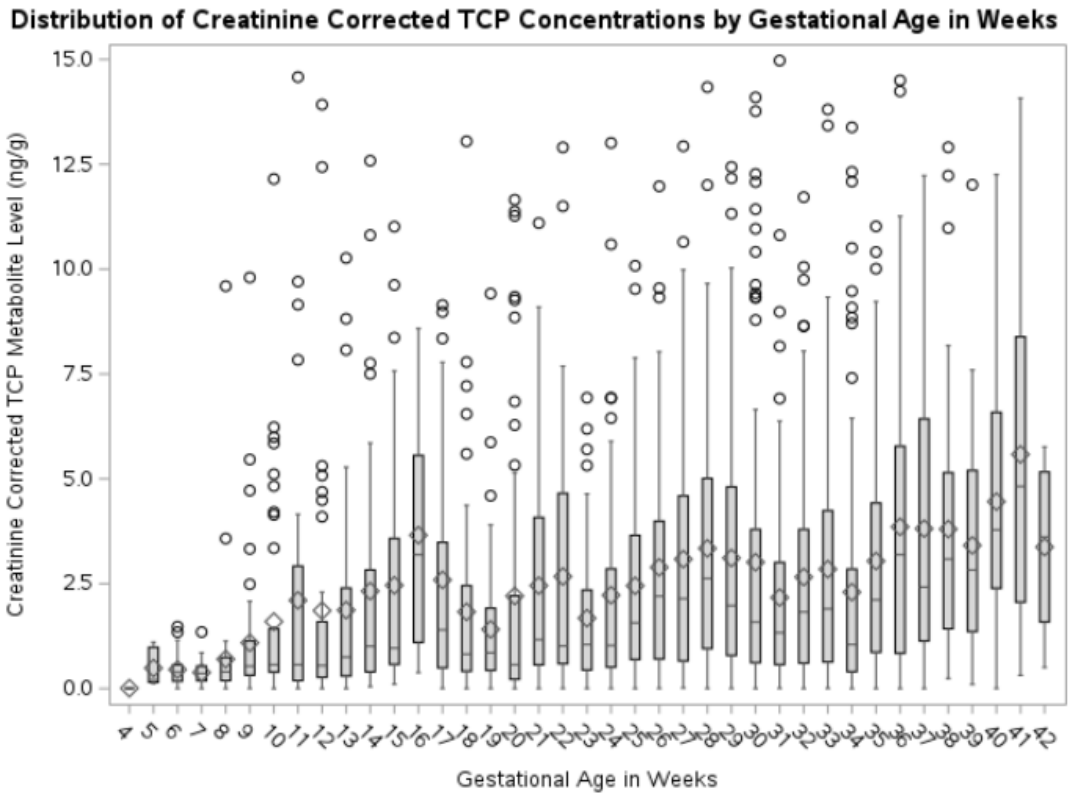


Figure 5.10. Distribution of TCP Concentrations by Gestational Age (n=1,362)

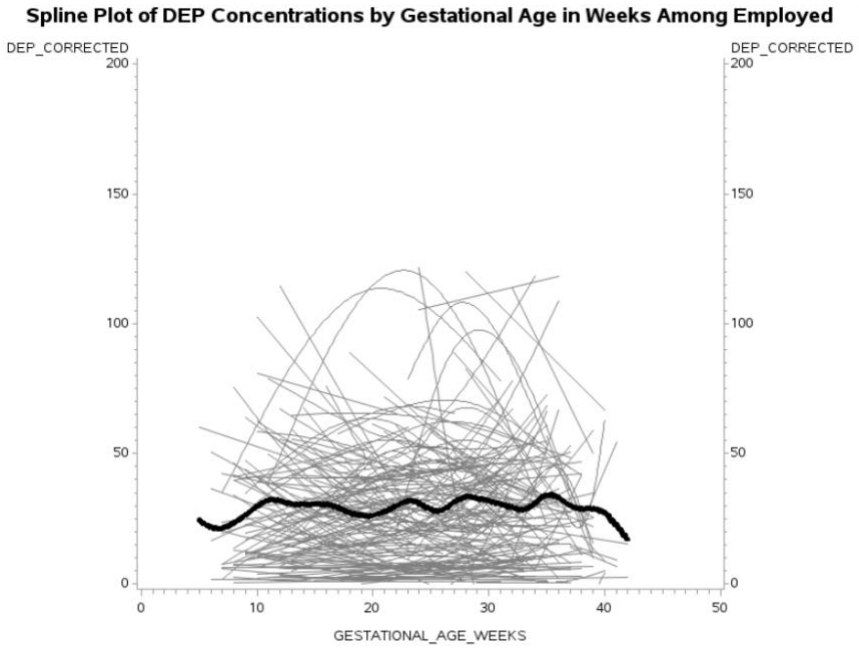


Figure 5.11. Spline Plot of DEP Concentrations by Gestational Age Among Employed

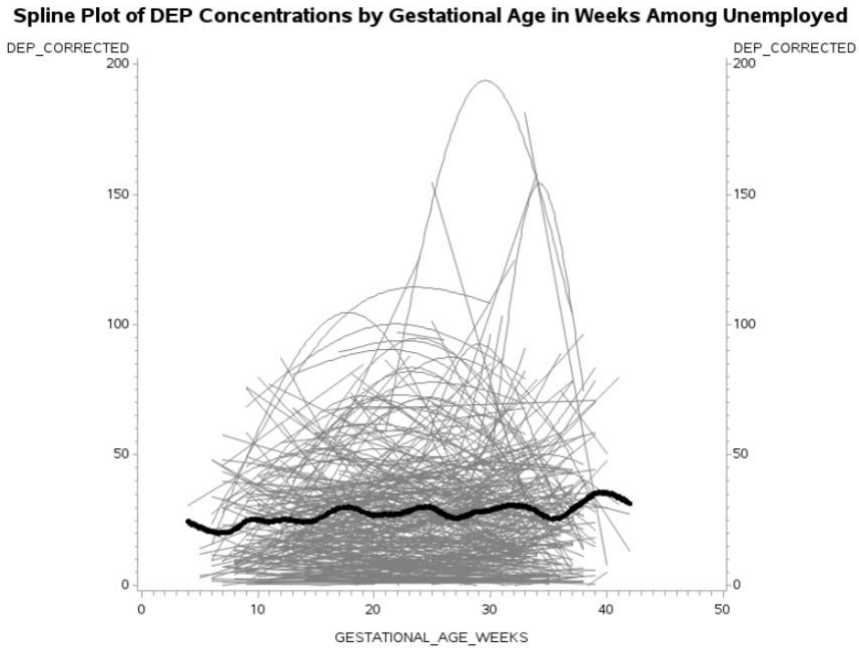


Figure 5.12. Spline Plot of DEP Concentrations by Gestational Age Among Unemployed

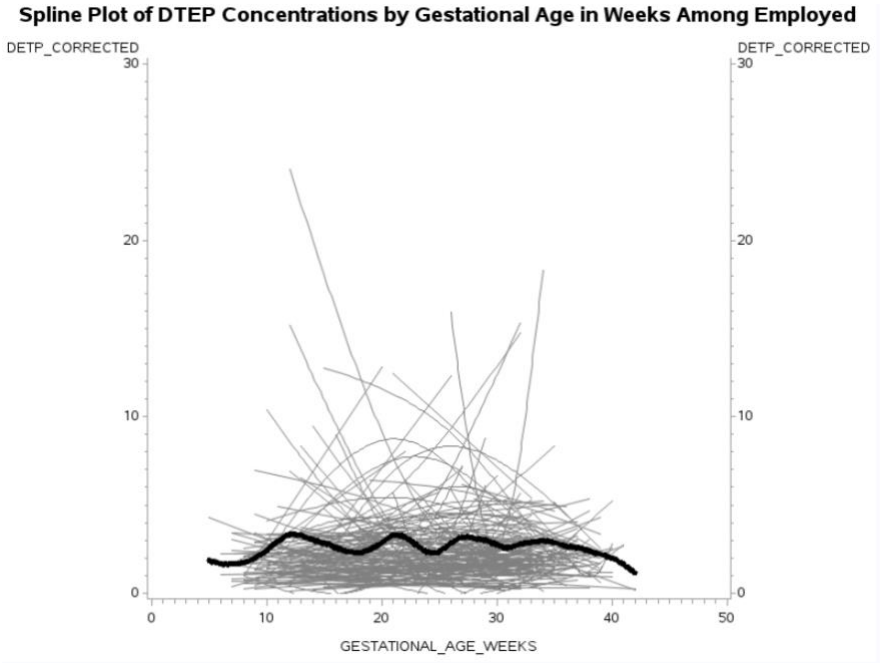


Figure 5.13. Spline Plot of DETP Concentrations by Gestational Age Among Employed

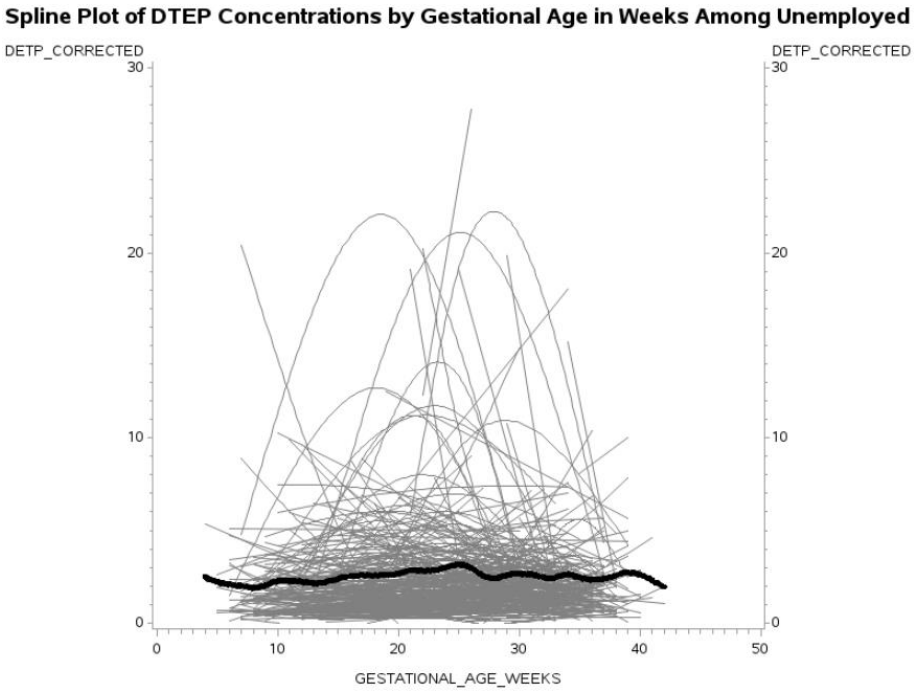


Figure 5.14. Spline Plot of DETP Concentrations by Gestational Age Among Unemployed

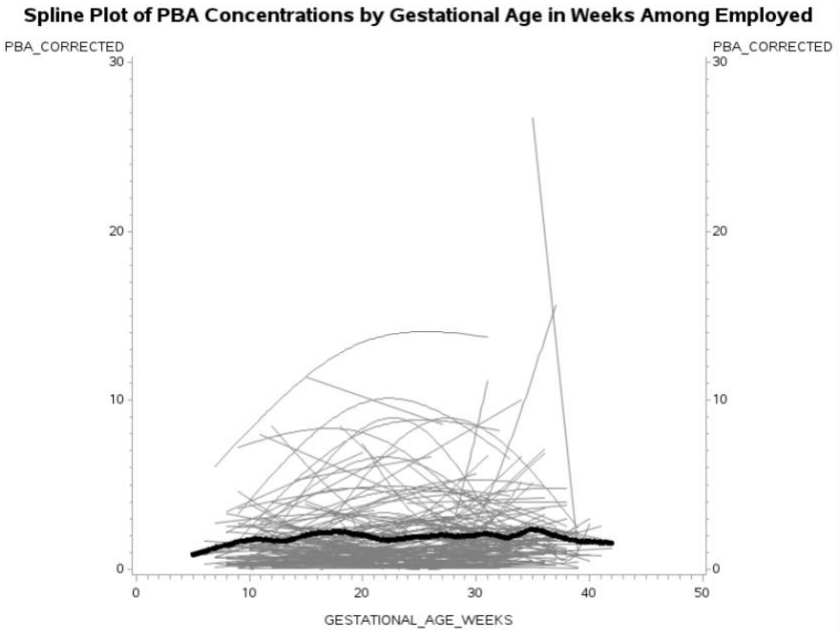


Figure 5.15. Spline Plot of PBA Concentrations by Gestational Age Among Employed

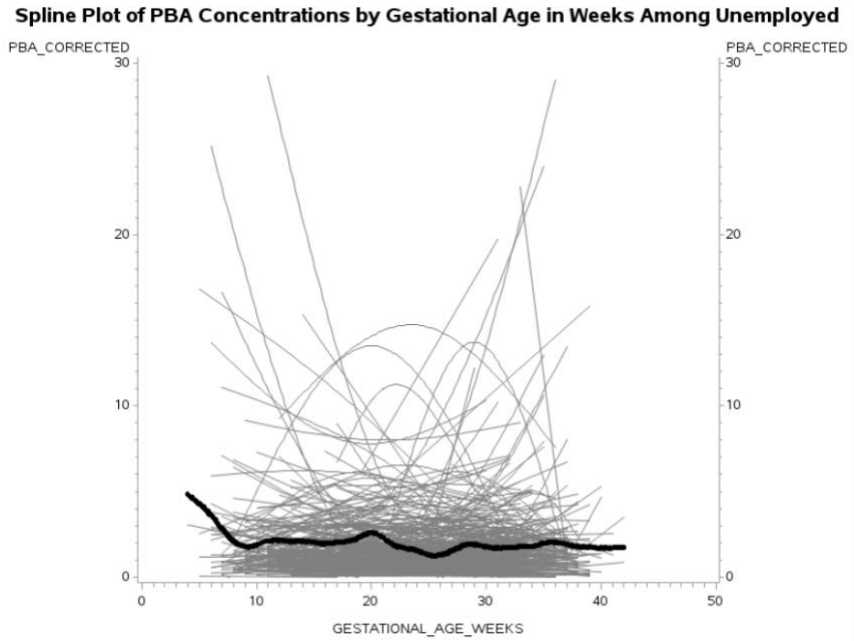


Figure 5.16. Spline Plot of PBA Concentrations by Gestational Age Among Unemployed

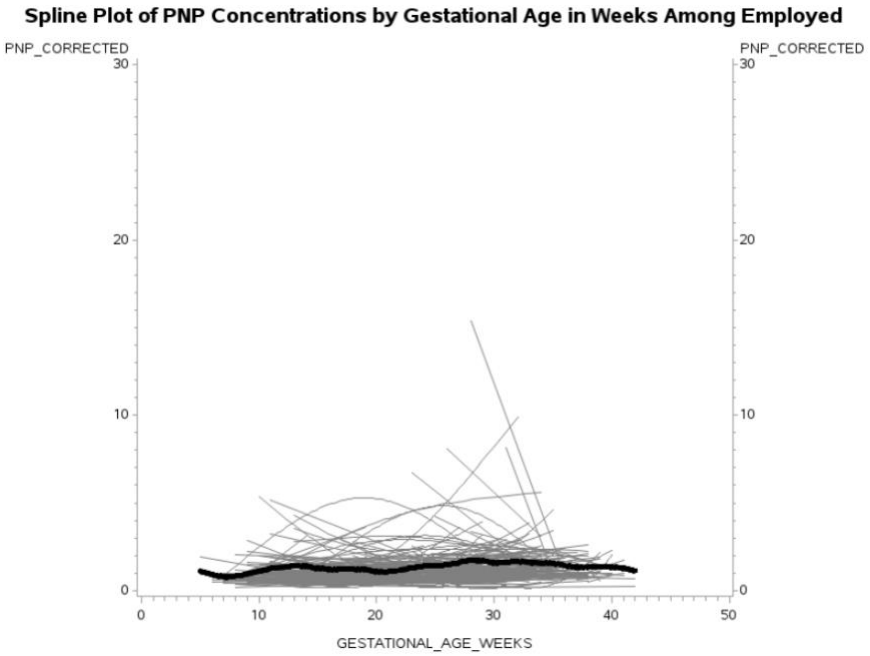


Figure 5.17. Spline Plot of PNP Concentrations by Gestational Age Among Employed

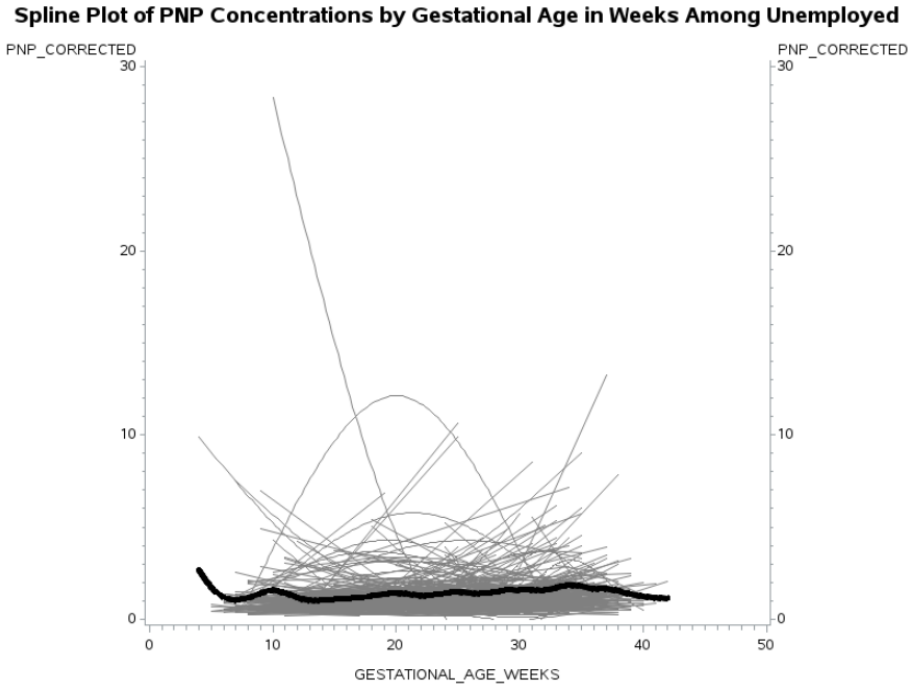


Figure 5.18. Spline Plot of PNP Concentrations by Gestational Age Among Unemployed

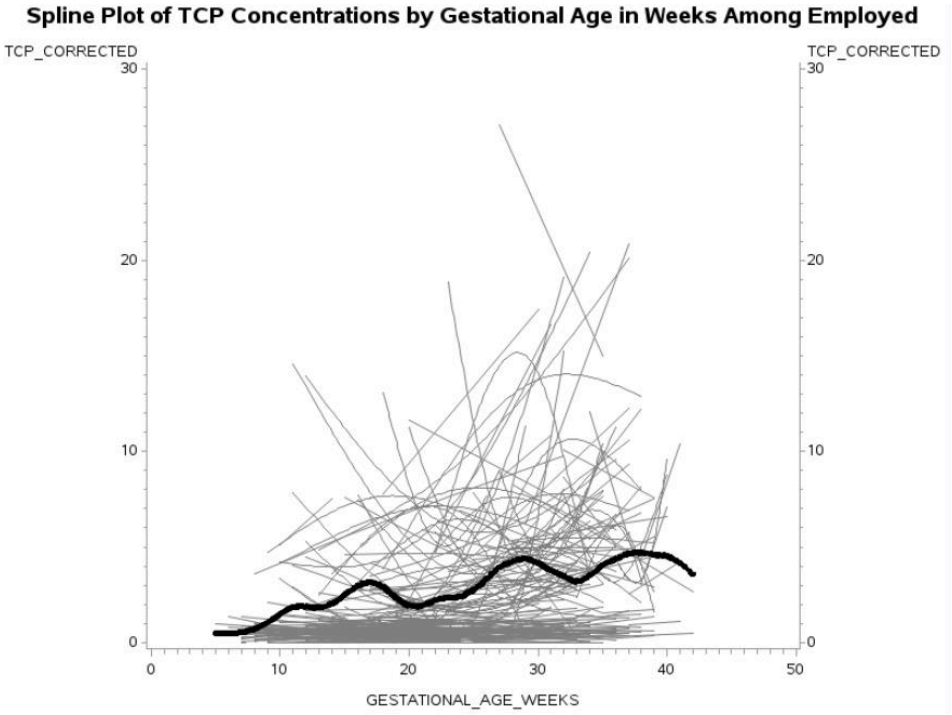


Figure 5.19. Spline Plot of TCP Concentrations by Gestational Age Among Employed

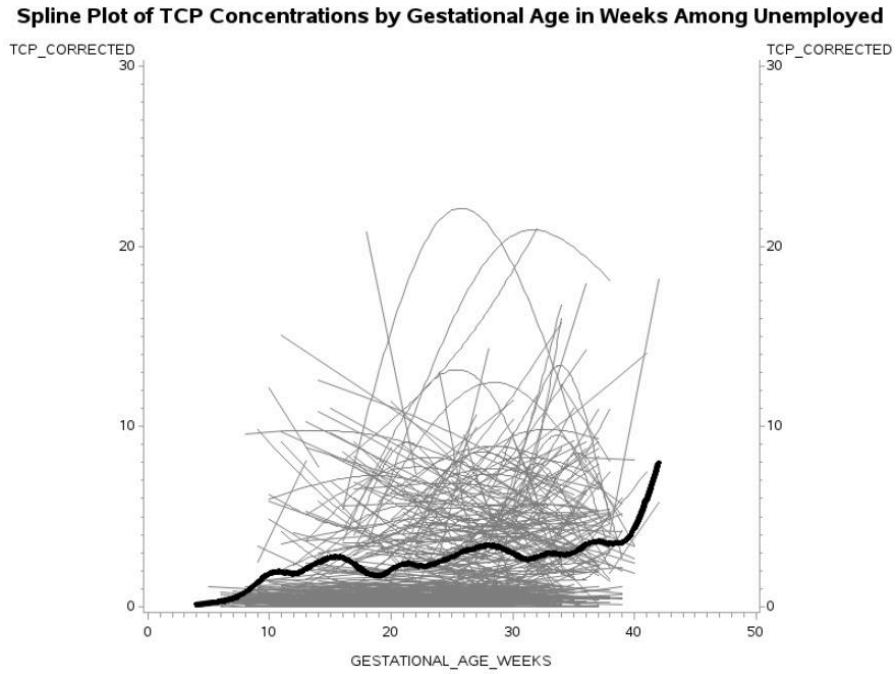


Figure 5.20. Spline Plot of TCP Concentrations by Gestational Age Among Unemployed

CHAPTER 6

ASSOCIATIONS BETWEEN ORGANOPHOSPHATE AND PYRETHROID EXPOSURE DURING PREGNANCY AND PRETERM BIRTH, ZIKA AND INFANTS IN PREGNANCY COHORT

[1]Mallis, N., Welton, M.., Knight, J., Shen, Y., Glenn, T., Cordero, JF

To be submitted to Environment International

Abstract

Background: Organophosphates (OPs) and pyrethroids (PYRs) are insecticides used worldwide in large scale farming, home applications, and in insect repellents. Humans can be exposed to OPs and PYRs through food and water intake, inhalation, and direct contact. Few studies have investigated associations between maternal prenatal exposure to OPs and PYRs and preterm birth (PTB). Studies that examined the risk of OPs and/or PYRs exposure during pregnancy and PTB report diverse risk outcomes from no risk to an increased risk, and some studies reporting exposure as protective.

Objective: Examine the association between organophosphate and pyrethroid urine biomarker levels during pregnancy and preterm birth using data from the Zika in Infants and Pregnancy (ZIP) cohort.

Methods: 1,326 pregnant persons were recruited from ZIP research sites in Puerto Rico (North Karst), Nicaragua (Managua), Brazil (Salvador), and Guatemala (Guatemala City). Participants were first interviewed about demographic characteristics and followed throughout pregnancy until delivery. Urine samples were collected at regular visits and analyzed for pesticide biomarker concentrations by Mount Sinai's Children's Health Exposure Analysis Resource (CHEAR). Seven OP biomarkers were measured: Diethyldithiophosphate (DEDP), Diethylphosphate (DEP), Diethylthiophosphate (DETP), Dimethyldithiophosphate (DMDP), Dimethylphosphate (DMP), Dimethylthiophosphate (DMTP), 3,5,6-trichloro-2- pyridinol (TCP), and three PYR biomarkers: 3-phenoxybenzoic acid (PBA), 4-fluoro-3-phenoxybenzoic acid (FPBA), 4-nitrophenol (PNP). Preterm birth was defined as livebirths born before 37 completed weeks of gestation. We calculated distributions for each metabolite stratified by preterm birth status. We used univariate and multivariable logistic regression to calculate crude and adjusted odds ratios for each metabolite and preterm birth.

Results: Both pyrethroid biomarkers (PBA and PNP) had protective associations against preterm birth. Both crude and adjusted models revealed that the odds of preterm birth were lower among those in the high PBA concentration level category compared with the low category [Crude OR (95% CI): 0.59 (0.37, 0.94)], [Adjusted OR (95% CI): 0.45 (0.24, 0.83)]. In the adjusted model for PNP, the odds were significantly lower among the medium concentration category compared to the low category [Adjusted OR (95% CI): 0.45 (0.26, 0.72)]. One organophosphate biomarker (TCP), showed a protective association with preterm birth with significant differences between high levels of TCP and low, but these were only apparent in the crude model ([Crude OR (95% CI): 0.53 (0.33, 0.85)]. The crude and adjusted logistic regression models did not reveal significant associations between the other two organophosphate biomarkers (DEP and DETP). All odds ratios were close to one and not statistically significant.

Conclusion/Discussion: Pyrethroid exposure displayed a protective association against preterm birth and one organophosphate biomarker (TCP) was also protective. The other two organophosphate metabolites (DEP and DETP) did not show a significant association. PYRs such as permethrin are used in mosquito nets and also sprayed directly on clothing to repel pests, which can lessen the chance of being bit or infected with vector-borne diseases. In our study, pyrethroid exposure/use may have reduced infection risk for zika and dengue, viruses associated with PTB.

Introduction

Organophosphates (OPs) and pyrethroids (PYRs) are both insecticides widely used across the world. Organophosphates are mostly applied in large scale farming, while pyrethroids are used in large scale farming, home applications, and in insect repellents that are sprayed directly on clothes and mosquito nets (2). OPs and PYRs came into prominence in the 1970's and were thought to be a safer option for pest control compared to organochlorines (DDT), which were banned in the US due to the deleterious effects on wildlife, persistence in the environment, and association with adverse health outcomes in humans (1). Common organophosphates pesticides include parathion, diazinon, malathion, and chlorpyrifos (2). Organophosphates poison insects and other pests by targeting acetylcholinesterase enzymes found in nerve endings, which causes organs to be overstimulated by excess acetylcholine and ultimately kills the insect via neurotoxicity (1). Permethrin, resmethrin, and sumithrin are common pyrethroids that are used around the world (2). Pyrethroids target the voltage-gated sodium channels in the nervous system in insects, causing paralysis and death (3). Both organophosphates and pyrethroids have been instrumental in advancing agriculture and public health efforts to combat vector borne diseases, but acute and long-term exposures have been associated with negative effects in humans (1).

Humans can be exposed to small amounts of OPs and PYRs through food and water intake, inhalation, and direct contact (1). Individuals who work in agriculture and other occupations that regularly use pesticides are at a higher risk of exposure (4). Similar to their intended effect on pests, OPs bring about toxic effects on humans by binding to the acetylcholinesterase enzyme and result in high levels of acetylcholine to build up. Both acute and long-term exposure can cause problems in the respiratory, cardiovascular, and nervous system

(2). Acute exposure to OPs can result in experiencing diarrhea, vomiting, vision issues, paralysis, and even death (1). Studies on long term exposure to OPs have revealed neurologic effects and associations with neurological diseases (17, 18). Several studies examining organophosphate urine biomarkers have found that OP exposure during pregnancy was associated with low birth weight, smaller head circumference, and smaller birth length (7-9). Existing literature also suggests that OP exposure during pregnancy is associated with neurodevelopmental and behavioral issues (10, 11). With pyrethroids, acute poisoning in humans can cause coughing and bronchospasm, nausea and vomiting (1). Studies have also suggested that pyrethroids have adverse effects on male sperm concentration and motility (19, 20). A recent study published in 2022 in found that first and second trimester exposures to high levels of PYRs were associated with poorer infant neurodevelopmental outcomes by age one (12). Few studies have examined OPs and PYRs effects on preterm birth (PTB).

Preterm birth is defined as babies who are born before 37 completed weeks of pregnancy (21). PTB is further classified into three different categories of preterm birth including extremely preterm (less than 28 weeks), very preterm (28 to 32 weeks), and moderate to late preterm (32 to 37 weeks). Preterm birth associated complications are the most common cause of death for children under 5 years old across the world (22). Short term complications can include lung, heart, gastrointestinal, and immune system conditions which may lead to long-term complications that can include cerebral palsy, learning disabilities, vision and hearing problems, and chronic health issues (23). A 2012 report using data from 184 countries estimated the global average preterm birth rate to be 11.1% (9.1-13.4) (24). In the US, preterm birth rates have been steadily increasing since 2015, when the preterm rate was reported to be 9.6% (24). The most recent data from the National Center for Health Statistics reported the US preterm birth rate in

2022 to be 10.38% (25). In Puerto Rico, one of the sites in our study, preterm birth rates have also been on the rise. In 2021, they reported a 11.77% PTB rate and 12.03% in 2022, higher than the US national rate (25). Preterm birth is an outcome with multiple known factors including maternal factors, uterine, and fetal factors (26). Among maternal factors, Infections during pregnancy, maternal diabetes, hypertension, and other maternal conditions are common (26). Uterine restriction as in the case of twins or higher number multiple pregnancies is another known cause (26). Among fetal conditions, large for gestational age is an example. Placental conditions such as placenta previa may also lead to preterm birth. Still, for most preterm births, the cause is unknown (21).

Long term and acute exposure to chemicals such as pesticides have been of interest as possible factors that impact preterm birth. A comprehensive meta-analysis of pesticide exposure during pregnancy published in 2023 found moderate evidence of an association between pesticide exposure and preterm birth (27). Further, the research team found several studies that suggest second and third trimester exposures to be the critical windows in which there is an increased risk for PTB (27). Research suggests that pesticides might act as endocrine disruptors and bring about changes in metabolism and fetal growth during pregnancy, which could lead to preterm birth (28). Phthalates are one type of endocrine disruptor and exposure to phthalates have been found to be associated with preterm birth and decreased gestation (29). Studies conducted among populations in Puerto Rico have found evidence that phthalate exposure during pregnancy is associated with preterm birth and mediated by testosterone, corticotrophin releasing hormone, and progesterone concentration levels (29). Further, they found these effects to only be apparent among males when stratifying by infant sex (29). Several studies using rats as subjects concluded that organophosphates prevent thyroid hormone-receptor binding, suggesting possible

endocrine disrupting effects (30). In vitro studies using pyrethroid biomarkers also suggest that the metabolites have estrogenic effects and possible endocrine disruption (31).

Studies that have examined the risk of OPs and/or PYRs exposure during pregnancy and PTB report diverse risk outcomes from no risk to an increased risk, and even some studies reporting exposure as protective. They also vary in study design, timepoint of data collection, and metabolites used to measure exposure, which makes drawing comparisons difficult. In 2022, Hu et. al. conducted an analysis using the China National Birth Cohort (n=302) and found that DEP urine concentration was associated with a higher risk of preterm birth (OR = 1.35, 95% CI = 1.11, 2.25) (34). Another cohort study in Bangladesh (n = 289) reported an increased risk for PTB among the high category 4-nitrophenol level as compared with the low (35). On the other hand, a 2021 study in Argentina (n=776) found no association between organophosphate exposure and preterm birth (36).

Several studies examined associations between organophosphate levels and gestational age, without quantifying the relationship between OPs and PTB. In 2022, Rauch et. al. concluded that urinary DAP concentrations were associated with shorter gestation (n=344) (37). Eskenazi et. al. also reported an association between organophosphate levels and shortened gestation in their 2004 study (n= 488), specifically with samples collected later in pregnancy (10). While this study reported the association with gestational age as the outcome, the overall preterm birth rate was low in the cohort. In 2012, Wang et. al. reported that log unit increase in DEP was associated with shorter gestation (n=187), although the relationship was only apparent among females (20). Suwannakul et. al. conducted a study in Thailand in 2021 (n=71) and found a significant negative association with DEP levels and gestational age (38). Finally, a study

conducted in Jordan in 2020 reported that DEDTP (n=104), an organophosphate biomarker, was negatively associated with gestational age (39).

Less studies have examined the relationship between pyrethroid biomarker concentrations and preterm birth. In 2020, Xu et. al. reported pyrethroid exposure to be significantly associated with a decreased risk for preterm birth (n=512) (40). A China based cohort study (n=545) conducted in 2015 found no association between pyrethroid biomarker concentration levels and preterm birth (41). Another study in Bangladesh (n=298) found no association between pyrethroid exposure and preterm birth (35).

Due to the importance of preterm birth as a global public health issue and the conflicting results among studies discussed above, our objective is to examine the association between organophosphate metabolites concentrations and pyrethroid metabolite concentrations and preterm birth among the Zika in Pregnancy in Infants and Pregnancy Cohort, which was motivated by the 2015-2016 outbreak of zika, a vector borne disease that was occurring at high rates in central and south America. Infants of pregnant persons infected with zika can be born with microcephaly. Participants in our study resided in Brazil, Guatemala, Nicaragua, and Puerto Rico, all places in which zika was a public health issue at the time of data collection (60)

Methods

Data used for this analysis was collected as part of the Zika in Infants and Pregnancy (ZIP) study. Details on the ZIP cohort are outlined previously in chapter 1 (62). Briefly, for our sub study, 1,326 pregnant persons were recruited from ZIP research teams in Puerto Rico (North Karst), Nicaragua (Managua), Brazil (Salvador), and Guatemala (Guatemala City). Women 15 years old or older who planned to give birth in the study regions and were not participating in

other research studies were deemed eligible. Participants were first interviewed in an initial visit for baseline demographics, medical history, and current pregnancy information. Participants then had monthly targeted clinical examinations where they were interviewed about occupational and household characteristics and other health behaviors. Pregnant persons enrolled in the study were followed through delivery and birth outcomes were recorded.

Biologic samples were collected at scheduled visits including blood and urine, used for Zika virus (ZIKV) analysis and specimen repositories. Additional urine samples were collected at visits for the pesticide analysis. Each participating site collected the urine samples and stored in -20-degree freezers in 15-50 ml conical tubes. Once the urine sample collection was completed, the samples were transported to the University of Georgia and stored in -20-degree freezers in 15-50 ml conical tubes. Samples were aliquoted into 5 ml vials before shipment to Mount Sinai's Children's Health Exposure Analysis Resource (CHEAR) for pesticide analysis.

CHEAR Pesticide Analysis

2,705 urine samples were sent to CHEAR and tested for organophosphate and pyrethroid pesticides. Seven biomarkers, Diethyldithiophosphate (DEDP), Diethylphosphate (DEP), Diethylthiophosphate (DETP), Dimethyldithiophosphate (DMDP), Dimethylphosphate (DMP), Dimethylthiophosphate (DMTP), and 3,5,6-trichloro-2- pyridinol (TCP). Three biomarkers, 3-phenoxybenzoic acid (PBA), 4-fluoro-3-phenoxybenzoic acid (FPBA), and 4-nitrophenol (PNP) were tested to detect pyrethroid levels. The Limit of Detection (LOD) values reported were the average of the daily LODs for the whole study. The daily LODs were used to identify the valid values for each sample. Quality control pairs were used to measure of precision based on the percent of the difference between duplicate sample concentrations relative to the mean of the

duplicate sample concentrations per subject. We merged the lab data from the CHEAR urine analysis with epidemiologic data from the ZIP study. Due to literature suggesting exposures later in pregnancy are most likely to be associated with preterm birth, we used the last collection from each participant in our analysis (27).

Exposure Ascertainment

Biomarkers with a limit of detection (LOD) greater than 70% were included in the study. For all organophosphate biomarkers, the LOD was 0.5 ng/mL. For all pyrethroid biomarkers, the LOD was 0.10 ng/mL. Out of all the OP biomarkers measured, DEP (98% detect) DETP (90% detect), and TCP (96% detect) had LODs greater than 70% and were therefore used as primary exposures in the analysis. Out of all the PYR biomarkers measured, PBA (90% detect) and PNP (96% detect), had LODs greater than 70% and were used as the primary exposures in this analysis. DEDP (8% detect), DMDP (6% detect), DMP (56% detect), DMTP (49% detect), and FPBA (17% detect) were below the 70% and excluded from our analysis. Among biomarkers that were included, samples with a concentration below the LOD were imputed using a standard formula in which the LOD is divided by the square root of two (54). Then, for each biomarker, concentration levels were divided by the creatinine level to correct for urine dilution [creatinine corrected level = metabolite level concentration / creatinine concentration] (53).

Before modeling, we categorized each creatinine corrected biomarker into low, medium, and high categories using tertiles calculated for each metabolite. The biomarker category levels were used as the main exposures in the models.

Outcome Ascertainment

The outcome of the study, preterm birth, was defined as babies being born alive before 37 completed weeks of gestation. Gestational age at delivery was calculated based on last menstrual period and delivery date. One hundred and sixty-seven (12.5%) participants missing information on preterm birth status were removed before analysis.

Statistical Analysis

We calculated means, standard deviations, geometric means, geometric standard deviations and percentiles for each creatinine corrected metabolite (DEP, DETP, TCP, PBA, and PNP) stratified by preterm birth status. We used t-tests to compare group means for each biomarker by preterm birth status. Due to skewness in the data, t-tests were run on the log of each creatinine corrected biomarker outcome. Descriptive statistics on the study population were calculated and stratified by preterm birth. Maternal characteristics included study site, maternal education, maternal age, marital status, smoking status, alcohol use, employment status, infant sex, pre-pregnancy weight, and pre-pregnancy BMI. We conducted chi-square tests for independence with each characteristic and the outcome. All tests were conducted at an alpha level of 0.05.

We calculated crude and adjusted logistic regression models for each creatinine corrected biomarker category level and preterm birth. The low level for each biomarker was used as the reference group. We present odds ratios and 95% confidence intervals for each biomarker category level. The adjusted models included site, age category, education level, pre-pregnancy BMI, marital status, smoking status, and sex of infant as covariates. Covariates were included in the adjusted model based upon prior knowledge of association with PTB, causal mapping, and maternal characteristics showing significant univariate associations with the outcome in our

sample. In order to further explore associations, we also ran the models with gestational age at birth as the outcome.

Results

After removing the 167 (12.5%) participants missing information on preterm birth, our sample included 1,159 pregnant persons (Table 6.1). The majority of the participants were under 25 years of age (54.01%), in the healthy BMI category (45.82%), and came from the Guatemala study site (37.53%). Most of the participants were either married or living with a partner (84%) and currently unemployed (70%). Forty-five percent of the participants did not have a high school diploma. Among those included in analysis, 122 (10.5%) experienced a preterm birth. The mean (SD) gestational age at which a preterm birth occurred in the sample was 35.1 (1.7) weeks and the earliest preterm birth in the study population occurred at 26 weeks. Among the participants who carried to full term, the mean (SD) gestational age at birth was 39.3 (1.3). As stated, this analysis used the last sample collection from each participant. Among those who experienced a preterm birth, the mean (SD) gestational age at sample collection was 27.79 (5.46) weeks. The mean (SD) gestational age at sample collection among those who carried full term was 31.4 (5.9) weeks.

Table 6.2 shows the distribution for each creatinine-corrected analyte stratified by preterm birth status. For both DEP and DETP, the geometric mean was higher among those who experienced a preterm birth compared with those who did not, but these differences were not significant at the 0.05 level (DEP geometric means - 19.43 ng/g creatinine in PTB group vs 17.43 ng/g creatinine in full term group, DETP geometric means- 2.07 ng/g creatinine in PTB group vs 1.9 ng/g creatinine in full term group). For TCP, the geometric mean was significantly

lower in the PTB group (0.98 ng/g creatinine in PTB group vs 1.39 ng/g creatinine full term group, p = 0.047).

For both pyrethroid biomarkers, the geometric mean was lower among those who experienced a preterm birth vs those who did not (PBA geometric means - 0.74 ng/g creatinine in PTB group vs 0.87 ng/g creatinine in full term group, PNP geometric means- 1.28 ng/g creatinine in PTB group vs 1.43 ng/g creatinine in full term group), but these differences were not significant.

When examining associations between maternal characteristics and preterm birth, we detected significant differences by site and sex of infant (Table 6.1). The proportion of preterm birth was highest among participants from Nicaragua (12.4%) and lowest among participants from Brazil (8.2%), p = 0.003. The proportion of preterm birth was higher among participants who gave birth to a male (11.63%) compared with those who gave birth to a female (8.68%), p <0.0001. There were no significant differences detected among the levels of the other maternal characteristics.

Organophosphate Biomarkers and Preterm Birth

The crude and adjusted logistic regression models did not reveal significant associations between DEP creatinine corrected concentration levels and preterm birth or DETP creatinine corrected concentration levels and preterm birth (Table 6.3 and Figure 6.1). All odds ratios were close to one and not statistically significant. The models with gestational age at birth as the outcome also did not show a significant relationship with DEP and DETP biomarker levels (Table 4). For the TCP biomarker, the odds of preterm birth were significantly lower among the

high TCP level category compared with the low category ([Crude OR (95% CI): 0.53 (0.33, 0.85)], but this association was null in the adjusted model.

Pyrethroid Biomarkers and Preterm Birth

All three pyrethroid biomarker levels displayed significant protective associations against preterm birth (Table 6.3 and Figure 6.2). The odds of preterm birth were lower among those in the high PBA level category compared with the low category and the association was significant in both the crude and adjusted models [Crude OR (95% CI): 0.59 (0.37, 0.94)], [Adjusted OR (95% CI): 0.45 (0.24, 0.83)]. In the crude model with PNP and preterm birth, the odds of preterm birth was lower among those in the high PNP level category compared with the low category [Crude OR (95% CI): 0.61 (0.39, 0.95)] and lower among those in the medium category compared with the low category [Crude OR (95% CI): 0.45 (0.28, 0.72)]. In the adjusted model for PNP, the odds were significantly lower among the medium category compared to the low category [Adjusted OR (95% CI): 0.45 (0.26, 0.72)]. Higher PBA levels were associated with lower gestational age at birth [Adjusted Beta (95% CI)= 1.3 (-2.17, -0.09)] compared with the low PBA level. Models with gestational age at birth as the outcome showed similar relationships with PBA (Table 4).

Discussion

In this study, we examined associations between three biomarkers for organophosphate exposures (DEP, DETP, and TCP) and preterm birth and two for pyrethroid exposures (PBA and PNP) and preterm birth among the ZIP cohort. Our analysis did not reveal significant associations between DEP or DETP biomarkers and preterm birth. All point estimate confidence

intervals for DEP category level and DETP category level were null in both the crude and adjusted models. For TCP, another organophosphate biomarker, higher levels were significantly associated with decreased risk for preterm birth in the crude model but not the adjusted. ([Crude OR (95% CI): 0.53 (0.33, 0.85)]. The few other studies that examine relationships between OP biomarkers and PTB have reported diverse risk outcomes with some finding that high OP biomarker levels are associated with PTB and others finding no association.

Both pyrethroid biomarkers (PBA and PNP) displayed protective associations against preterm birth. Both crude and adjusted models revealed that the odds of preterm birth were lower among those in the high PBA level category compared with the low category [Crude OR (95% CI): 0.59 (0.37, 0.94)], [Adjusted OR (95% CI): 0.45 (0.24, 0.83)]. In the adjusted model for PNP, the odds were significantly lower among the medium category compared to the low category [Adjusted OR (95% CI): 0.45 (0.26, 0.72)]. Existing research on PYRs urine biomarkers and preterm birth have also suggested protective associations with PTB or no association. In 2020, Xu et. al. also reported pyrethroid exposure to be significantly associated with a decreased risk for preterm birth (n=512) (40).

Exposure to organophosphate and pyrethroid pesticides mainly occurs due to home usage, occupational use, and in the case of pyrethroids, direct contact on the clothing. Common PYRs such as permethrin are used in mosquito nets and also sprayed directly on clothing to repel pests, which can lessen the chance of being bit or infected with vector-borne diseases. Dengue and zika virus are two vector-borne diseases that have displayed associations with preterm delivery (63). In our study, pyrethroid use may have reduced infection risk for Zika, dengue, and other viruses associated with PTB. This might explain the protective associations for pyrethroid biomarker and PTB that we report in the results of our study.

Another possible route of human exposure to pesticides is through food intake, specifically fruits and vegetables that can contain small amounts of OPs or PYRs (64). Studies show that pregnant persons who lead healthier lifestyles that include a balanced diet are at a lower risk for preterm birth (65). This could perhaps explain our results with regard to pyrethroid biomarkers displaying protective associations with preterm birth. Participants in our study who ate more fruits and vegetables might have been less likely to deliver preterm due to eating a healthier diet and living an overall healthier lifestyle. Our findings might be more of a representation of fruit/vegetable consumption and healthy habits, but we cannot say this with confidence because information on dietary intake among participants was not available.

The original cohort study from which this data was sourced was motivated by the 2015-2016 outbreak of zika, a vector borne disease that was occurring at high rates in central and south America. Infants of pregnant persons infected with zika can be born with microcephaly. Participants in our study resided in Brazil, Guatemala, Nicaragua, and Puerto Rico, all places in which zika was a public health issue at the time of data collection (60). The results of this study should be interpreted within the context of the zika outbreak. During urine specimen collection, participants might have used insecticides in their homes, yards, and on their clothing more frequently than previous years.

We present a comprehensive analysis of several urine metabolite biomarkers and their associations with PTB using a large, diverse cohort, but there are some limitations to consider when interpreting the results. First, data was not available on certain covariates that would have been of interest. Information on dietary (fruit and vegetable) intake, type of occupation (agriculture vs other types of employment), and pesticide usage habits would have been helpful in the analysis and interpretation of the results. Future studies conducted on pesticide exposure

and preterm birth include this information on participants. We were also missing information on our outcome, preterm birth, among 167 (12.5%) of the participants. Measuring urine biomarker levels during pregnancy provides an indication of pesticide exposure, but it is important to note that these measurements reflect recent exposures that would have occurred within a few days of the sample collection (43). Our sample collections might not have detected high exposures that could have occurred several days or weeks after. Last, while we were able to obtain information on a large sample of pregnant persons from four different countries, the results might not be generalizable to other populations outside of the Americas.

While our analysis did not find evidence of increased risk for preterm birth associated with OP and PYR exposure, other studies have found a relationship and we recommend this to be further investigated in future cohorts. Subsequent studies on OP and PYR metabolites and preterm birth should additionally test for hormone concentration levels at the time of sample collection. Since OPs and PYRs have displayed possible endocrine disrupting capabilities, this information might be important. Mediation analysis could reveal changes in testosterone, corticotrophin releasing hormone, and progesterone concentration levels due to OP and PYR exposure that could lead to preterm birth.

Table 6.1. Characteristics of the study sample (n=1,159)

Characteristic	Full Term (n=1,037)	Preterm (n=122)	p-value[a]
Maternal age at enrollment			p = 0.65
<25	566 (53.62)	70 (57.38)	
25-30	288 (27.77)	33 (27.05)	
>30	193 (18.61)	19 (15.57)	
Site			p = 0.003
Brazil-Salvador	260 (25.07)	16 (13.11)	
Guatemala- Guatemala City	393 (37.90)	42 (34.43)	
Nicaragua- Managua	139 (13.4)	24 (19.67)	
Puerto Rico-North Karst	245 (23.63)	40 (32.79)	
Maternal Education			p = 0.061
Less than a High School Diploma	482 (46.48)	56 (45.9)	
High School Diploma or Equivalent	377 (32.50)	29 (2.77)	
Associate Degree, Bachelor's Degree or Higher	216 (20.83)	37 (30.33)	
Missing	2 (0.19)	0 (0.00)	
Pre-Pregnancy BMI Category (Kilograms/Height^2)			p = 0.97
Underweight ($\leq$ 18.5)	98 (9.45)	12 (9.84)	
Healthy (18.5 $<$ 24.9)	478 (46.09)	53 (43.44)	
Overweight (25 $<$ 29.9)	265 (25.55)	34 (27.87)	
Obese ($\geq$ 30)	183 (17.65)	21 (17.21)	
Missing	13 (1.25)	2 (1.64)	
Marital Status			p = 0.051
Single	173 (16.68)	11 (9.02)	
Married	318 (30.67)	32 (26.63)	
Living Together, Not Married	543 (52.36)	79 (64.75)	
Missing	3 (0.29)	0 (0.00)	
Employment Status			p = 0.80
Currently Employed	242 (23.34)	27 (22.13)	
Not Currently Employed	722 (69.62)	88 (72.13)	
Missing	7 (5.74)	73 (7.04)	
Smoking Status			p = 0.37
Never Smoked	827 (79.75)	91 (74.59	
Former Smoker	82 (7.19)	11 (9.02)	
Current Smoker	6 (0.58)	2 (1.64)	
Missing	122 (11.76)	18 (14.75)	
Alcohol Use			p = 0.08
Never	688 (66.35)	88 (72.13)	
Before Pregnancy	158 (15.24)	9 (7.38)	
During Pregnancy	108 (10.41)	17 (13.93)	
Missing	83 (8.00)	8 (6.56)	
Sex of Infant			p < 0.0001
Female	505 (48.7)	48 (39.34)	
Male	532 (51.3)	70 (57.38)	
Missing	0 (0.00)	4 (3.28)	

[a]p-values were calculated from chi-square tes

Table 6.2. Distribution of creatinine corrected DEP, DETP, PBA, PNP, TCP in the urine (ng/mg creatinine) at last visit before pregnancy in the ZIP Cohort by preterm birth.

							Percentile							
Analyte	Preterm Status	N	AM	SD	GM	GSD	10th	25th	Median	75th	90th	95th	Max	p- value
														p = 0.304
DEP	Full Term	1,037	28.72	26.3	17.43	0.66	3.47	9.75	23.55	39.70	60.29	76.53	202.53	
	Preterm	122	30.46	26.95	19.43	1.91	4.7	9.10	25.14	44.02	60.62	72.96	161.43	
														p = 0.32
DETP	Full Term	1,037	2.82	5.42	1.90	0.05	0.67	1.18	1.98	3.18	5.20	7.21	144.16	
	Preterm	122	3.10	3.62	2.07	0.16	0.72	1.11	2.11	3.51	6.74	8.54	27.76	
														p = 0.16
PBA	Full Term	1,037	2.44	20.23	0.87	0.04	0.02	0.42	0.99	2.01	4.25	6.24	10.56	
	Preterm	122	1.36	1.69	0.74	0.08	0.15	0.35	0.79	1.53	3.02	4.93	10.32	
														p = 0.47
PNP	Full Term	1,037	1.67	1.78	1.34	0.03	0.65	0.91	1.31	2.00	2.89	3.72	40.87	
	Preterm	122	1.83	2.70	1.28	0.08	0.68	0.84	1.05	1.76	2.87	5.73	25.9	
														p = 0.047***
TCP	Full Term	1,037	3.48	6.00	1.39	0.07	0.28	0.63	1.77	4.33	8.13	11.32	120.4	
	Preterm	122	2.89	4.55	0.98	0.16	0.18	0.54	0.98	2.80	8.49	12.08	28.22	

Note: DEP, Diethylphosphate; DETP, Diethylthiophosphate; PBA, 3-phenoxybenzoic acid; PNP, 4-nitrophenol; TCP, 3,5,6-trichloro-2-pyridinol, AM, arithmetic mean; SD, arithmetic standard deviation; GM, geometric mean; GSD, geometric standard deviation

Table 6.3. Odds ratios by level of last urinary DEP, DETP, PBA, PNP, and TCP collection concentrations measured during pregnancy and preterm birth.

	Crude		**Adjusted[a]**
Analyte	OR (95% CI)		OR (95% CI)
DEP Urine Concentrations			
High	1.15 (0.72, 1.59)		1.17 (0.78, 1.96)
Medium	1.00 (0.62 1.59)		0.85 (0.50, 1.46)
Low (Reference)	-		-
DETP Urine Concentrations			
High	1.18 (0.66, 1.69)		1.33 (0.78, 2.27)
Medium	1.06 (0.75, 1.86)		1.10 (0.64, 1.90)
Low (Reference)	-		
TCP Urine Concentrations			
High	**0.53 (0.33, 0.85)***		1.1 (0.53, 2.13)
Medium	0.70 (0.45, 1.09)		1.00 (0.57, 1.78)
Low (Reference)	-		-
PBA Urine Concentrations			
High	**0.59 (0.37, 0.94)***		**0.45 (0.24, 0.83)***
Medium	0.74 (0.47, 1.15)		0.66 (0.39, 1.27)
Low (Reference)	-		-
PNP Urine Concentrations			
High	**0.61 (0.39, 0.95)***		0.61 (0.36, 1.03)
Medium	**0.45 (0.28, 0.72)***		**0.45 (0.26, 0.79)***
Low (Reference)	-		-

[a] Adjusted for site, age category, education level, pre-pregnancy BMI, marital status, smoking status, and sex of infant

Table 6.4. Beta coefficients and 95% confidence intervals by level of last urinary DEP, DETP, PBA, PNP, and TCP collection concentrations measured during pregnancy and preterm birth.

	Univariate		Adjusted[a]
Analyte	Beta (95% CI)		Beta (95% CI)
DEP Urine Concentrations			
High	0.18 (-1.1,0.74)		0.21 (-1.11, 0.69)
Medium	0.29 (-1.1,0.74)		0.08 (-0.82, 0.98)
Low (Reference)	-		-
DETP Urine Concentrations			
High	0.4 (-0.5, 1.13)		0.09 (-0.99, 0.81)
Medium	0.11 (-1.03, 0.81)		0.32 (-1.2,0.61)
Low (Reference)	-		
TCP Urine Concentrations			
High	3.4 (2.5, 4.3)		-0.75 (-2.0, 0.5)
Medium	1.38 (0.49, 2.27)		-0.4 (-1.46, 0.66)
Low (Reference)	-		-
PBA Urine Concentrations			
High	-0.23 (-1.15, 0.69)		**-1.3 (-2.17, -0.09)***
Medium	0.65 (-0.28, 1.58)		-0.01 (-1.0, 0.97)
Low (Reference)	-		-
PNP Urine Concentrations			
High	0.37 (-0.5, 1.3)		0.23 (-0.67, 1.24)
Medium	0.20 (-0.71, 1.11)		0.08 (-0.86, 1.03)
Low (Reference)	-		-

[a] Adjusted for site, age category, education level, pre-pregnancy BMI, marital status, smoking status, and sex of infant

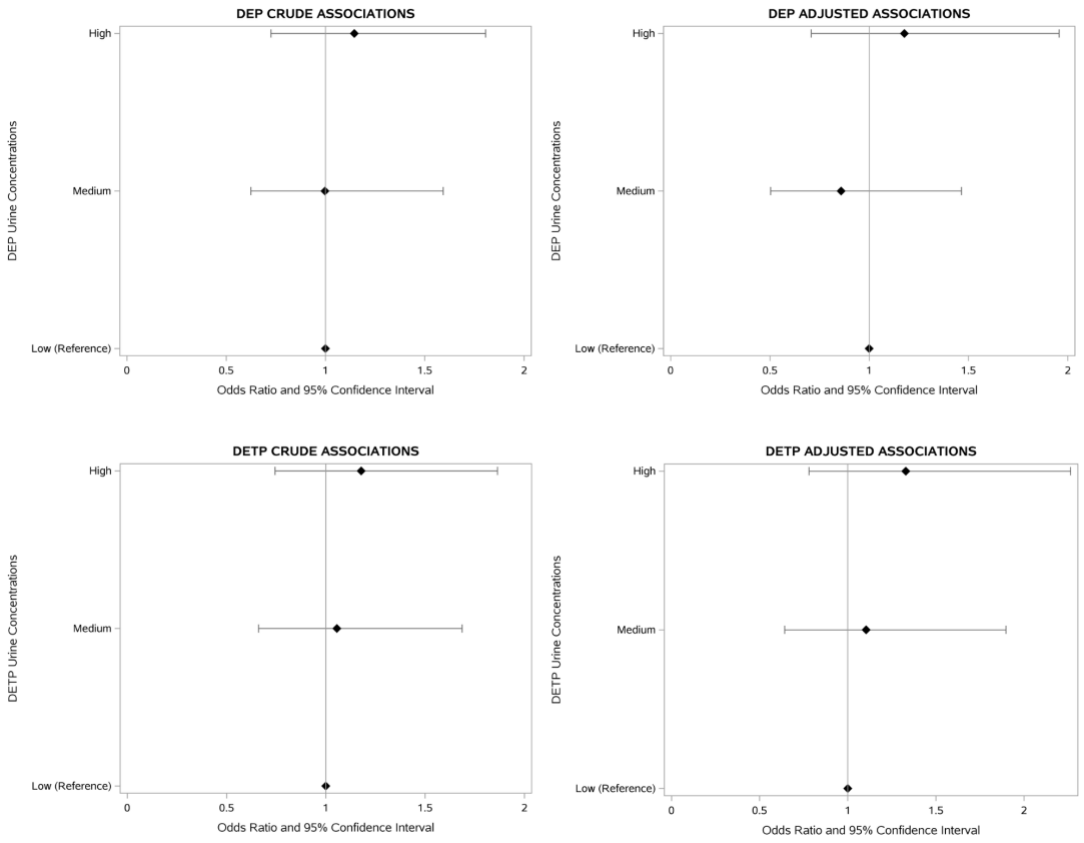


Figure 6.1. Crude and adjusted odds ratios for DEP and DETP concentration levels and preterm birth

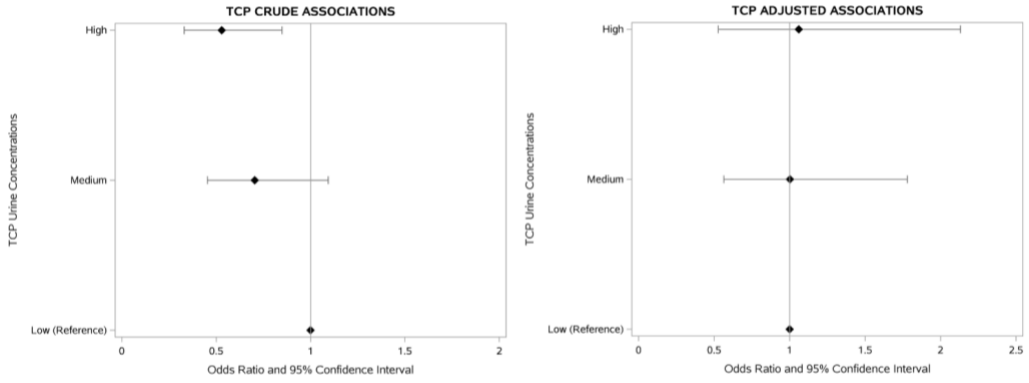


Figure 6.2. Crude and adjusted odds ratios for TCP concentration levels and preterm birth

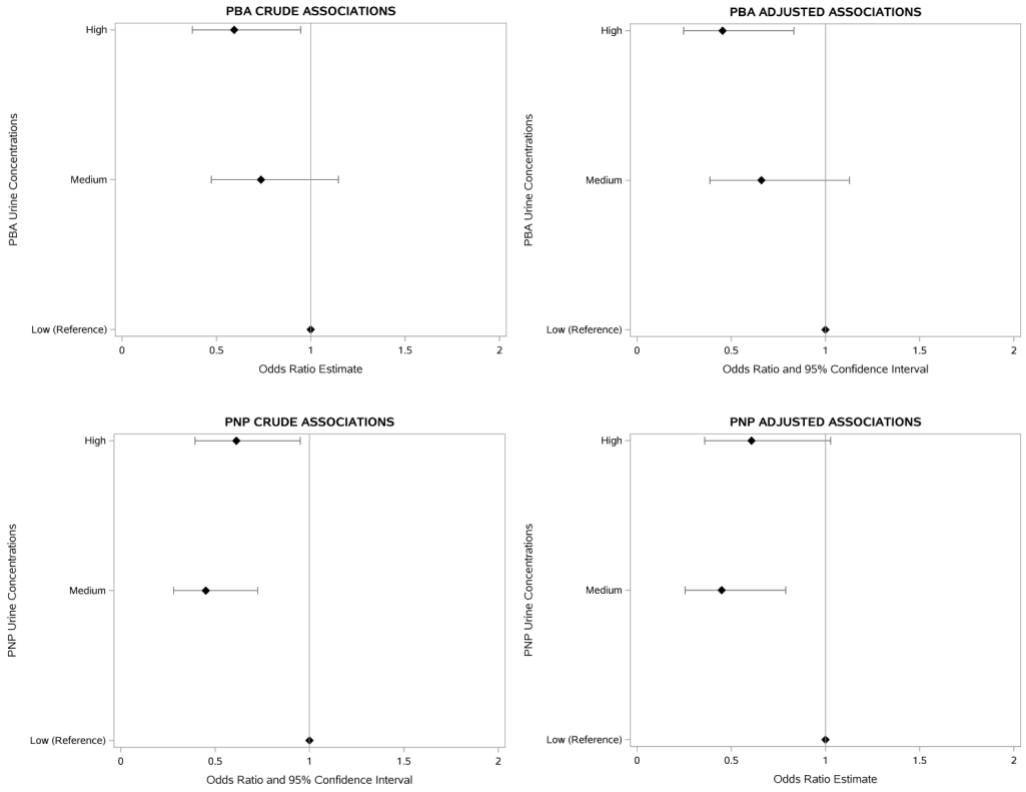


Figure 6.3. Crude and adjusted odds ratios for PBA and PNP concentration levels and preterm birth

CHAPTER 7

CONCLUSION

Our analysis adds to the growing body of knowledge on pesticide exposure during pregnancy. The first aim results revealed significant differences in organophosphates and pyrethroid metabolite levels by site in the Zika and Infants in Pregnancy Cohort. Participants from Brazil had the highest geometric mean levels of TCP and DEP metabolites and Nicaragua had the highest DETP levels. For pyrethroids, participants from Guatemala had the highest PNP levels and Nicaragua had the highest PBA levels. Primary water source, BMI category, education level, marital status, and locality were all significantly associated with organophosphate metabolite levels. DEP and DETP levels were higher in those who reported consuming bottle/delivery water vs city water and lower in obese vs healthy weight. DETP levels also varied by education level (lower in college vs less than high school), locality (higher in downtown vs suburban), and first pregnancy (higher among first pregnancy group). TCP levels varied by water source (higher in well water vs city), marital status (higher married or living together), and education level (lower in college vs less than high school). Some studies have found that runoff from pesticide use in agriculture and home applications might transfer to surface and ground water treated for drinking use, which should be an important exposure route to study in future research. Runoff from pesticide use in agriculture and home applications can transfer to surface and ground water treated for drinking use (56). A 2021 study conducted in Costa Rica examined pesticide occurrence and water quality and found detections of chlorpyrifos, an organophosphate

pesticide, in both water and sediment samples (57). They identified chlorpyrifos and other pesticides as possible factors in lower water quality in microcatchments near the sample collections (57). The geometric DEP an DETP mean levels in our study were higher than some previous studies conducted in the same regions, which could be explained by possible higher use of pesticides due to the Zika outbreak in 2015-2016. Our second aim examined how pesticide levels were changing across gestation. Among the five metabolites examined in this study, TCP was the only analyte that appeared to be increasing throughout gestation. This could also be due to increased usage of home pesticides due to the increases in zika cases during the years in which our study took place. This could also just be a reflection of higher usage in certain months of the year. In our third aim, two metabolites (PNP and PBA) of pyrethroid exposure displayed a protective association against preterm birth, and one organophosphate (TCP) also displayed a protective association. PYRs such as permethrin are used in mosquito nets and also sprayed directly on clothing to repel pests, which can lessen the chance of being bit or infected with vector-borne diseases. TCP is also biomarker for chlorpyrifos, an organophosphate used in structural pest control for mosquitos. In our study, pyrethroid and OP exposure/use may have reduced infection risk for zika and dengue, viruses associated with PTB.

Given the associations revealed between water source and OP metabolite levels from our first aim, we recommend future studies that not only test for urine levels in the subjects, but also in the source from which they draw their water for drinking and cooking. Comparisons between subject metabolite levels and levels in the water between groups could reveal more information about this association. In our analysis, TCP (an organophosphate) seemed to increase with gestational age while other metabolites remained stable. Future research with this dataset and other cohorts should examine this relationship and take into account seasonality to identify

higher months of exposure. Further, studies should collect data on direct usage of organophosphates and pyrethroids in populations who work directly with pesticides in agriculture or gardening to examine how exposure profiles change over time. Future studies conducted on maternal characteristics associated with pesticide exposure should also include dietary intake information on participants and pesticide usage habits that will likely impact urine biomarker levels. While our analysis did not find evidence of increased risk for preterm birth associated with OP and PYR exposure, other studies have found a relationship and we recommend this to be further investigated in future cohorts. Subsequent studies on OP and PYR metabolites and preterm birth should additionally test for hormone concentration levels at the time of sample collection. Since OPs and PYRs have displayed possible endocrine disrupting capabilities, this information might be important. Mediation analysis could reveal changes in testosterone, corticotrophin releasing hormone, and progesterone concentration levels due to OP and PYR exposure that could lead to preterm birth.

REFERENCES

1. Koureas M, Tsakalof A, Tsatsakis A, Hadjichristodoulou C. Systematic review of biomonitoring studies to determine the association between exposure to organophosphorus and pyrethroid insecticides and human health outcomes. Toxicol Lett. 2012 Apr 25;210(2):155-68.
2. Simaremare SRS, Hung CC, Hsieh CJ, Yiin LM. Relationship between Organophosphate and Pyrethroid Insecticides in Blood and Their Metabolites in Urine: A Pilot Study. Int J Environ Res Public Health. 2019 Dec 18;17(1).
3. Field LM, Emyr Davies TG, O'Reilly AO, Williamson MS, Wallace BA. Voltage-gated sodium channels as targets for pyrethroid insecticides. Eur Biophys J. 2017;46(7):675-9.
4. Damalas CA, Koutroubas SD. Farmers' Exposure to Pesticides: Toxicity Types and Ways of Prevention. Toxics. 2016 Jan 8;4(1).
5. Morgan MK. Dietary Pyrethroid Exposures and Intake Doses for 188 Duplicate-Single Solid Food Items Consumed by North Carolina Adults. Toxics. 2020 Jan 22;8(1).
6. Jara EA, Winter CK. Safety levels for organophosphate pesticide residues on fruits, vegetables, and nuts. International Journal of Food Contamination. 2019 2019/08/10;6(1):6.
7. Perera FP, Rauh V, Tsai WY, Kinney P, Camann D, Barr D, et al. Effects of transplacental exposure to environmental pollutants on birth outcomes in a multiethnic population. Environ Health Perspect. 2003 Feb;111(2):201-5.
8. Balalian AA, Liu X, Herbstman JB, Daniel S, Whyatt R, Rauh V, et al. Prenatal exposure to organophosphate and pyrethroid insecticides and the herbicide 2,4-dichlorophenoxyacetic acid and size at birth in urban pregnant women. Environ Res. 2021 Oct;201:111539.
9. Whyatt RM, Rauh V, Barr DB, Camann DE, Andrews HF, Garfinkel R, et al. Prenatal Insecticide Exposures and Birth Weight and Length among an Urban Minority Cohort. Environ Health Perspect. 2004;112(10):1125-32.
10. Eskenazi B, Harley K, Bradman A, Weltzien E, Jewell NP, Barr DB, et al. Association of in utero organophosphate pesticide exposure and fetal growth and length of gestation in an agricultural population. Environ Health Perspect. 2004 Jul;112(10):1116-24.
11. Engel SM, Berkowitz GS, Barr DB, Teitelbaum SL, Siskind J, Meisel SJ, et al. Prenatal organophosphate metabolite and organochlorine levels and performance on the Brazelton Neonatal Behavioral Assessment Scale in a multiethnic pregnancy cohort. Am J Epidemiol. 2007 Jun 15;165(12):1397-404.
12. Qi Z, Song X, Xiao X, Loo KK, Wang MC, Xu Q, et al. Effects of prenatal exposure to pyrethroid pesticides on neurodevelopment of 1-year- old children: A birth cohort study in China. Ecotoxicology and Environmental Safety. 2022 2022/04/01/;234:113384.
13. Dórea JG. Exposure to environmental neurotoxic substances and neurodevelopment in children from Latin America and the Caribbean. Environmental Research. 2021 2021/01/01/;192:110199.
14. Froes Asmus CI, Camara VM, Landrigan PJ, Claudio L. A Systematic Review of Children's Environmental Health in Brazil. Ann Glob Health. 2016 Jan-Feb;82(1):132-48.
15. Alavanja MC. Introduction: pesticides use and exposure extensive worldwide. Rev Environ Health. 2009 Oct-Dec;24(4):303-9.

16. Panis C, Kawassaki ACB, Crestani APJ, Pascotto CR, Bortoloti DS, Vicentini GE, et al. Evidence on Human Exposure to Pesticides and the Occurrence of Health Hazards in the Brazilian Population: A Systematic Review. Front Public Health. 2021;9:787438.
17. Hancock DB, Martin ER, Mayhew GM, Stajich JM, Jewett R, Stacy MA, et al. Pesticide exposure and risk of Parkinson's disease: A family-based case-control study. BMC Neurology. 2008 2008/03/28;8(1):6.
18. Rohlman DS, Anger WK, Lein PJ. Correlating neurobehavioral performance with biomarkers of organophosphorous pesticide exposure. NeuroToxicology. 2011 2011/03/01/;32(2):268-76.
19. Elbetieha A, Da'as SI, Khamas W, Darmani H. Evaluation of the Toxic Potentials of Cypermethrin Pesticide on Some Reproductive and Fertility Parameters in the Male Rats. Archives of Environmental Contamination and Toxicology. 2001 2001/11/01;41(4):522-8.
20. Wang P, Tian Y, Wang XJ, Gao Y, Shi R, Wang GQ, et al. Organophosphate pesticide exposure and perinatal outcomes in Shanghai, China. Environ Int. 2012 Jul;42:100-4.
21. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet. 2008 Jan 5;371(9606):75-84.
22. Beck S, Wojdyla D, Say L, Betran AP, Merialdi M, Requejo JH, et al. The worldwide incidence of preterm birth: a systematic review of maternal mortality and morbidity. Bull World Health Organ. 2010 Jan;88(1):31-8.
23. Torchin H, Ancel PY. [Epidemiology and risk factors of preterm birth]. J Gynecol Obstet Biol Reprod (Paris). 2016 Dec;45(10):1213-30.
24. Blencowe H, Cousens S, Oestergaard MZ, Chou D, Moller AB, Narwal R, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet. 2012 Jun 9;379(9832):2162-72.
25. Hamilton BEM, Joyce A.;Osterman, Michelle;. Births: Provisional Data for 2022. National Center for Health Statistics (US). 2023;NVSS vital statistics rapid release ; report no. 28.
26. Richard E. Behrman ASB. PRETERM BIRTH CAUSES, CONSEQUENCES, AND PREVENTION; 2007.
27. Lin S, Li J, Yan X, Pei L, Shang X. Maternal pesticide exposure and risk of preterm birth: A systematic review and meta-analysis. Environ Int. 2023 2023/08/01/;178:108043.
28. Kolan AS, Hall JM. Association of Preterm Birth and Exposure to Endocrine Disrupting Chemicals. Int J Mol Sci. 2023 Jan 19;24(3).
29. Cathey AL, Aung MT, Watkins DJ, Rosario ZY, Vélez Vega CM, Alshawabkeh AN, et al. Mediation by hormone concentrations on the associations between repeated measures of phthalate mixture exposure and timing of delivery. J Expo Sci Environ Epidemiol. 2022 May;32(3):374-83.
30. McKinlay R, Plant JA, Bell JNB, Voulvoulis N. Endocrine disrupting pesticides: Implications for risk assessment. Environ Int. 2008 2008/02/01/;34(2):168-83.
31. Garey J, Wolff MS. Estrogenic and Antiprogestagenic Activities of Pyrethroid Insecticides. Biochemical and Biophysical Research Communications. 1998 1998/10/29/;251(3):855-9.
32. Silver MK, Fernandez J, Tang J, McDade A, Sabino J, Rosario Z, et al. Prenatal Exposure to Glyphosate and Its Environmental Degradate, Aminomethylphosphonic Acid

(AMPA), and Preterm Birth: A Nested Case-Control Study in the PROTECT Cohort (Puerto Rico). Environ Health Perspect. 2021 May;129(5):57011.
33. Eaton JL, Cathey AL, Fernandez JA, Watkins DJ, Silver MK, Milne GL, et al. The association between urinary glyphosate and aminomethyl phosphonic acid with biomarkers of oxidative stress among pregnant women in the PROTECT birth cohort study. Ecotoxicol Environ Saf. 2022 Mar 15;233:113300.
34. Hu P, Li H, Vinturache A, Tian Y, Pan C, Hu Y, et al. Urinary organophosphate metabolite concentrations and birth sizes among women conceiving through in vitro fertilization in Shanghai, China. Environ Res. 2022 Feb 28;211:113019.
35. Jaacks LM, Diao N, Calafat AM, Ospina M, Mazumdar M, Ibne Hasan MOS, et al. Association of prenatal pesticide exposures with adverse pregnancy outcomes and stunting in rural Bangladesh. Environ Int. 2019 Dec;133(Pt B):105243.
36. Cecchi A, Alvarez G, Quidel N, Bertone MC, Anderle S, Sabino G, et al. Residential proximity to pesticide applications in Argentine Patagonia: impact on pregnancy and newborn parameters. Environmental Science and Pollution Research. 2021 2021/10/01;28(40):56565-79.
37. Rauch SA, Braun JM, Barr DB, Calafat AM, Khoury J, Montesano AM, et al. Associations of prenatal exposure to organophosphate pesticide metabolites with gestational age and birth weight. Environ Health Perspect. 2012 Jul;120(7):1055-60.
38. Suwannakul B, Sapbamrer R, Wiwattanadittakul N, Hongsibsong S. Organophosphate Pesticide Exposures in Early and Late Pregnancy Influence Different Aspects of Infant Developmental Performance. Toxics. 2021 Apr 30;9(5).
39. Suwannakul B, Sapbamrer R, Wiwattanadittakul N, Hongsibsong S. Prenatal organophosphate exposure can cause adverse birth outcomes to humans. Environ Sci Pollut Res Int. 2021 Sep;28(33):45064-74.
40. Xu Q, Zhu B, Dong X, Li S, Song X, Xiao X, et al. Pyrethroid pesticide exposure during early pregnancy and birth outcomes in southwest China: a birth cohort study. J Toxicol Sci. 2020;45(5):281-91.
41. Ding G, Cui C, Chen L, Gao Y, Zhou Y, Shi R, et al. Prenatal exposure to pyrethroid insecticides and birth outcomes in Rural Northern China. J Expo Sci Environ Epidemiol. 2015 May;25(3):264-70.
42. Davies JE, Peterson JC. Surveillance of occupational, accidental, and incidental exposure to organophosphate pesticides using urine alkyl phosphate and phenolic metabolite measurements. Ann N Y Acad Sci. 1997 Dec 26;837:257-68.
43. Lu C, Bravo R, Caltabiano LM, Irish RM, Weerasekera G, Barr DB. The presence of dialkylphosphates in fresh fruit juices: implication for organophosphorus pesticide exposure and risk assessments. J Toxicol Environ Health A. 2005 Feb 13;68(3):209-27.
44. Bradman A, Eskenazi B, Barr DB, Bravo R, Castorina R, Chevrier J, et al. Organophosphate urinary metabolite levels during pregnancy and after delivery in women living in an agricultural community. Environ Health Perspect. 2005 Dec;113(12):1802-7.
45. Donauer S, Altaye M, Xu Y, Sucharew H, Succop P, Calafat AM, et al. An Observational Study to Evaluate Associations Between Low-Level Gestational Exposure to Organophosphate Pesticides and Cognition During Early Childhood. Am J Epidemiol. 2016 Sep 1;184(5):410-8.
46. van den Dries MA, Pronk A, Guxens M, Spaan S, Voortman T, Jaddoe VW, et al. Determinants of organophosphate pesticide exposure in pregnant women: A population-based cohort study in the Netherlands. International Journal of Hygiene and Environmental Health. 2018 2018/04/01/;221(3):489-501.

47. Lewis RC, Cantonwine DE, Anzalota Del Toro LV, Calafat AM, Valentin-Blasini L, Davis MD, et al. Distribution and determinants of urinary biomarkers of exposure to organophosphate insecticides in Puerto Rican pregnant women. Sci Total Environ. 2015 Apr 15;512-513:337-44.
48. Muñoz-Quezada MT, Iglesias V, Lucero B, Steenland K, Barr DB, Levy K, et al. Predictors of exposure to organophosphate pesticides in schoolchildren in the Province of Talca, Chile. Environ Int. 2012 Oct 15;47:28-36.
49. Llop S, Murcia M, Iñiguez C, Roca M, González L, Yusà V, et al. Distributions and determinants of urinary biomarkers of organophosphate pesticide exposure in a prospective Spanish birth cohort study. Environ Health. 2017 May 17;16(1):46.
50. Lewis RC, Cantonwine DE, Anzalota Del Toro LV, Calafat AM, Valentin-Blasini L, Davis MD, et al. Distribution and determinants of urinary biomarkers of exposure to organophosphate insecticides in Puerto Rican pregnant women. Science of The Total Environment. 2015 2015/04/15/;512-513:337-44.
51. Berman T, Goldsmith R, Göen T, Spungen J, Novack L, Levine H, et al. Urinary concentrations of organophosphate pesticide metabolites in adults in Israel: Demographic and dietary predictors. Environ Int. 2013 2013/10/01/;60:183-9.
52. Buck Louis GM, Yeung E, Kannan K, Maisog J, Zhang C, Grantz KL, et al. Patterns and Variability of Endocrine-disrupting Chemicals During Pregnancy: Implications for Understanding the Exposome of Normal Pregnancy. Epidemiology. 2019 Nov;30 Suppl 2(Suppl 2):S65-s75.
53. Allen R, Mage D, Gondy G, Christensen C, Barr D, Needham L. THE USE OF A CREATININE CORRECTION FOR REPORTING CHILDREN'S URINARY PESTICIDE CONCENTRATIONS. Epidemiology. 2004;15(4).
54. Hornung RW, Reed LD. Estimation of Average Concentration in the Presence of Nondetectable Values. Applied Occupational and Environmental Hygiene. 1990 1990/01/01;5(1):46-51.
55. Suwannarin N, Prapamontol T, Isobe T, Nishihama Y, Nakayama SF. Characteristics of Exposure of Reproductive-Age Farmworkers in Chiang Mai Province, Thailand, to Organophosphate and Neonicotinoid Insecticides: A Pilot Study. Int J Environ Res Public Health. 2020 Oct 27;17(21).
56. Syafrudin M, Kristanti RA, Yuniarto A, Hadibarata T, Rhee J, Al-Onazi WA, et al. Pesticides in Drinking Water-A Review. Int J Environ Res Public Health. 2021 Jan 8;18(2).
57. Ramírez-Morales D, Pérez-Villanueva ME, Chin-Pampillo JS, Aguilar-Mora P, Arias-Mora V, Masís-Mora M. Pesticide occurrence and water quality assessment from an agriculturally influenced Latin-American tropical region. Chemosphere. 2021 2021/01/01/;262:127851.
58. Sokoloff K, Fraser W, Arbuckle TE, Fisher M, Gaudreau E, LeBlanc A, et al. Determinants of urinary concentrations of dialkyl phosphates among pregnant women in Canada - Results from the MIREC study. Environ Int. 2016 Sep;94:133-40.
59. Gerchman F, Tong J, Utzschneider KM, Zraika S, Udayasankar J, McNeely MJ, et al. Body mass index is associated with increased creatinine clearance by a mechanism independent of body fat distribution. J Clin Endocrinol Metab. 2009 Oct;94(10):3781-8.
60. Plourde AR, Bloch EM. A Literature Review of Zika Virus. Emerg Infect Dis. 2016 Jul;22(7):1185-92.

61. Rauh VA, Garfinkel R, Perera FP, Andrews HF, Hoepner L, Barr DB, et al. Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. Pediatrics. 2006 Dec;118(6):e1845-59.
62. Lebov JF, Arias JF, Balmaseda A, Britt W, Cordero JF, Galvão LA, et al. International prospective observational cohort study of Zika in infants and pregnancy (ZIP study): study protocol. BMC Pregnancy Childbirth. 2019 Aug 7;19(1):282.
63. O'Kelly B, Lambert JS. Vector-borne diseases in pregnancy. Ther Adv Infect Dis. 2020 Jan-Dec;7:2049936120941725.
64. Curl CL, Porter J, Penwell I, Phinney R, Ospina M, Calafat AM. Effect of a 24-week randomized trial of an organic produce intervention on pyrethroid and organophosphate pesticide exposure among pregnant women. Environ Int. 2019 2019/11/01/;132:104957.
65. Gete DG, Waller M, Mishra GD. Prepregnancy dietary patterns and risk of preterm birth and low birth weight: findings from the Australian Longitudinal Study on Women's Health. The American Journal of Clinical Nutrition. 2020 2020/05/01/;111(5):1048-58.