Investigating Agricultural Exposures, Inflammation, and Child Respiratory Health in the Lower Yakima Valley of Washington

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Abstract

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Background: Exposure to organophosphate (OP) pesticides, a common class of insecticides used in the Lower Yakima Valley of Washington, has been linked to asthma outcomes and biomarkers in children, potentially through immunological pathways; however, mechanistic understanding of this relationship is limited. Measurement of a profile of cytokines in biological media is a powerful tool for gleaning pathophysiological insight about these respiratory responses to these agricultural exposures. Additionally, cytokines provide a perspective through which to assess temporal trends in both OP pesticide exposures and inflammatory responses, a consideration particularly important for agricultural communities with unique seasonal variability in exposure profiles.

Methods: This study aims to 1) assess cytokine variability over time and across a population of children in an OP-intensive agricultural region of Washington and 2) explore relationships between low-level OP exposure and inflammatory responses related to respiratory health, notably asthma. To achieve these aims, dialkylphosphate metabolites were measured in urine and

a profile of cytokines was measured in plasma throughout three agricultural seasons in 2011 for children in the University of Washington Center for Child Environmental Health Risks Research (CHC) cohort. Bayesian mixed effects models fit via Markov chain Monte Carlo Hamiltonian sampling methods were leveraged to assess seasonal and demographic variability while linear mixed effects regressions and logistic binomial regressions were used to explore relationships between OP exposure, inflammation, and asthma status.

Results: Through these methods, significant seasonal variability was found for levels of IFN-γ and IL-8 in child plasma for this agricultural cohort; however, no significant associations were discerned between OP exposure, inflammation, and asthma.

Conclusion: This work illustrates how strategically defining systems-based cytokine profiles provides a perspective through which to gain mechanistic insight through epidemiological methods. These results highlight the importance of harnessing seasonal granularity for understanding environmental determinants of inflammation and respiratory health and that other methods of investigating these relationships may be underestimating some of the variability at play.

Acknowledgments

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BACKGROUND

1. CHILD RESPIRATORY HEALTH

Asthma, a chronic respiratory condition, is a common affliction in the United States (US) with the prevalence rate for children (<18 y) estimated at 6.2%, amounting to over 4.5 million cases.[1] While phenotypically diverse, asthma is typically associated with airway inflammation, remodeling, and hyperresponsiveness.[2,3] Children living with an asthma diagnosis experience symptoms such as wheeze, cough, dyspnea, and chest tightness.[4]

A recent comprehensive literature review and meta-analysis published in 2025 outlined general risk factors associated with childhood asthma.[5] The identified factors include family history of asthma, older age, male sex, and parental smoking. In addition to these risk factors, other environmental determinants of asthma have been recognized. Associations between certain environmental exposures (e.g. traffic and power plant related air pollution) and childhood asthma are well-established; however, recent preliminary evidence has begun to implicate other exposures (e.g. organophosphate pesticides, plasticizers) as drivers of asthma pathogenesis.[2,6,7,8] Another 2024 systematic review addresses respiratory-associated risk factors related to the home environment.[9] The results highlight the substantial body of evidence supporting the relationship between proximity to urban centers and respiratory diseases. This is thought to be due to the high levels of particulate matter (PM), CO, NOx, SO_2, and volatile organic compounds (VOC) exposure relating to urban sources such as fuel combustion. In 2013, Yakima county, an agricultural community in Washington, reported one of the highest rates of asthma hospitalizations and mortality in the state.[10] This disparity is notable as these communities are far removed from urban centers associated with many of the aforementioned risk factors.

Considering physiological and behavioral features unique to childhood (e.g. frequent hand-to-mouth activity, increased respiratory rate), early-life is considered a period of heightened susceptibility to environmental exposures.[7] Since it is evident in the described literature that environmental exposures are key risk factors in the pathogenesis of childhood respiratory conditions, understanding the biological responses to early-life exposure is essential for effective protection of respiratory health.

2. IMMUNE AND INFLAMMATORY SIGNALING

Cytokines, signaling molecules facilitating immune and inflammatory responses, play key roles in the pathogenesis of inflammatory conditions including asthma and other respiratory diseases. With recent advancements in quantification techniques and a growing understanding of the complex, context-dependent signaling pathways, measuring a profile of cytokine levels in biological media has become a powerful tool for characterizing unique patterns of inflammation.[11] Moreover, cytokines' propensity to respond to an array of dynamic exposures and their role as mediators of immune and inflammatory health makes them compelling biomarkers through which to consider both exposure and disease.

Unfortunately, capturing the dynamics of their signaling cascade and appropriately interpreting measured levels has proven to be challenging. This difficulty is attributed to individual cytokines' overlapping, context-dependent functions and their transience in circulation; however, utilizing a systems-based perspective and measuring a comprehensive profile of multiple cytokines along a signaling pathway, rather than individual characteristic cytokines of those pathways, may provide an opportunity to further our understanding of the intricacies of these cascades in relation to a system-specific health outcome.[11] This provides an ideal lens through which to consider chronic inflammatory conditions such as asthma.

Table 1 provides an overview of literature evidence connecting a profile of cytokines to respiratory associated signaling pathways. Using this systems-based perspective, Figure 1 summarizes the immune cell origin of these pathways as they relate to respiratory health outcomes highlighting the complex, context-dependent nature of these signaling cascades.

Table 1. Summary of existing literature evidence addressing the connection between cytokine signaling pathways and respiratory health

Cytokine	Respiratory Health Related Findings	Methods	Reference
IL-1β	IL-1β signaling pathway is upregulated in pediatric asthma cases	RNA sequencing of peripheral blood samples from asthmatic and healthy children	Wei et al., 2021[12]
	IL-1β enhances production of IL-6 and IL-8 from airway epithelial cells	In-vitro assessment of transformed human tracheal epithelial cell lines incubated with IL-1β and TNF-α to assess production of IL-6 and IL-8	Cromwell et al., 1992[13]
	Pro-inflammatory, IL-1β is associated with barrier dysfunction at the airway epithelium	In-vitro characterization of barrier function for human primary airway cells	Coyne et al., 2002[14]

IL-4	IL-4 levels elevated in children with asthma and wheeze	Longitudinal birth cohort study in Salinas Valley, CA; measurement of IFN-γ and IL-4 in blood samples	Duramad et al., 2006[15]
	Increased IL-4 levels in children with asthma	Case-control study assessing serum levels of IL-4 and IFN-γ for children with and without asthma	Lama et al., 2011[16]
	IL-4 levels elevated in children with wheeze	Case-control study assessing cytokine expression in child serum from the Xiangya Hospital in China with and without wheeze	Tang et al., 2022[17]
	IL-4 induces of differentiation of Th9 cells; pathway is upregulated in cases with wheeze	Case-control study assessing cytokine levels and immune cell populations in peripheral blood for infants with and without wheeze from the Binhai County Hospital in China	Liu et al., 2020[18]
IL-5	IL-5, a cytokine secreted by Th2 cells, regulates eosinophil proliferation and function	Eosinophils separated from the whole blood of allergic human subjects co-cultured with cytokines and characterized	Sedgwick et al., 1995[19]
	Positive association between IL-5 and atopic asthma	Longitudinal birth cohort study of children between 8 and 18 years old; in-vitro assessment of peripheral blood mononuclear cells immune response to dust mite challenge	Heaton et al., 2005[20]
IL-6	Airway epithelial cells produce IL-6	In-vitro assessment of transformed human tracheal epithelial cell lines incubated with IL-1β and TNF-α to assess production of IL-6 and IL-8	Cromwell et al., 1992[13]
	IL-6 is a precursor to Th17 differentiation	Mouse model assessing cytokine signaling cascades involved in Th17 differentiation	Zhou et al., 2007[21]
IL-8	Airway epithelial cells produce IL-8, a cytokine implicated in recruitment of neutrophils	Human bronchial epithelium and endothelial cell culture to assess IL-8 production and function	Marshall et al., 2001[22]
IL-17A	IL-17A, a neutrophil attractant and an inducer of IL-8 release from airway epithelial cells, is elevated in asthmatic patients	Case-control study assessing mRNA expression of cytokines in sputum from asthmatic and healthy adults	Bullens et al., 2006[23]
	IL-17A levels elevated in children with asthma and wheeze	Case-control study assessing cytokine expression in child serum from the Xiangya Hospital in China with and without wheeze	Tang et al., 2022[17]
TNF-α	Pro-inflammatory TNF-α is associated with barrier dysfunction at the airway epithelium	In-vitro characterization of barrier function for human primary airway cells	Coyne et al., 2002[14]
IFN-γ	Decreased IFN-γ levels in children with asthma and wheeze	Longitudinal birth cohort study in Salinas Valley, CA; measurement of IFN-γ and IL-4 in blood samples	Duramad et al., 2006[15]
	Decreased IFN-γ levels in children with asthma	Case-control study assessing serum levels of IL-4 and IFN-γ for children with and without asthma	Lama et al., 2011[16]

MCP-1	MCP-1 acts along eosinophilic pathways potentially playing a role in allergic inflammation	In-vitro assessment of human venous blood eosinophils and MCP-1 production	Izumi et al., 1997[24]

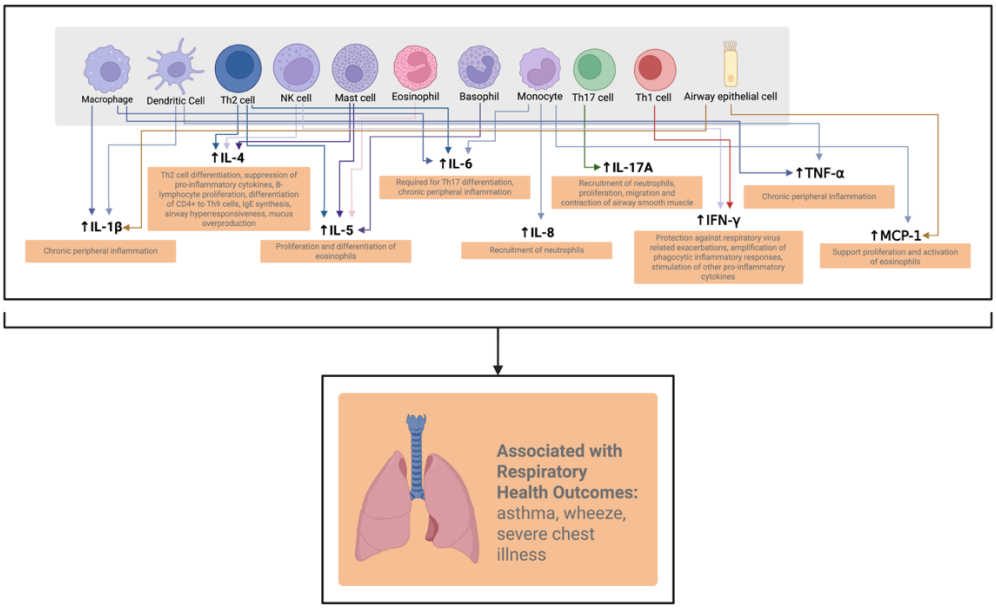

Figure 1. Schematic of cytokine functions as they relate to respiratory health and immune cell type.[25,26,27] Figure created with BioRender.com.

These studies have leveraged a variety of frameworks to tease out the role of individual cytokines within immune and inflammatory signaling pathways.[3,15,28] These frameworks include 1) comparing levels of signaling through pro- and anti-inflammatory pathways, 2) assessing the balance between Type 1 (Th1), Type 2 (Th2), and, more recently, Type 17 (Th17) T helper cell signaling, and 3) characterizing neutrophilic versus eosinophilic activity. Considering literature evidence, Table 2 outlines how a profile of respiratory-associated cytokines fits within the aforementioned frameworks.

Table 2. Frameworks of immune and inflammatory signaling implicated in respiratory health and examples of cytokines characteristic of those pathways

	IL-1β	**IL-4**	**IL-5**	**IL-6**	**IL-8**	**IL-17A**	**IFN-γ**	**TNF-α**	**MCP-1**
Respiratory	X	X	X	X	X	X	X	X	X
Neutrophilic				X	X	X			
Eosinophilic			X						X
Th1							X	X	
Th2		X	X						
Th17				X		X			
Pro-inflammatory	X			X			X	X	
Anti-inflammatory		X							

3. ORGANOPHOSPHATE (OP) PESTICIDES

3.1 Use and Trends

Agriculture is an essential component of the Washington state economy with pome fruits such as apples and pears dominating production.[29] According to data from the National Agriculture Statistics Service, Washington is consistently identified as the primary apple producer in the US making up 67% of total production in 2023.[29] In fact, the annual value of the Washington apple industry was estimated at around 1.991 billion US dollars in 2023.[29]

To curb pest driven crop damage and bolster production, the agricultural industry in the US uses pesticides at a scale of over one billion pounds per year as reported by the US Environmental Protection Agency (US EPA).[30] In 2012, insecticide use accounted for 5% of the pesticide market at an annual usage rate of over 60 million pounds.[30] Organophosphates (OPs), a class of insecticides, have been central to US agriculture making up 71% of total insecticide use in the year 2000.[30]

In 1996, the Food Quality Protection Act (FQPA) was passed as an addendum to the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food Drug and Cosmetic Act (FFDCA).[31] While FIFRA and FFDCA initially authorized the US EPA to regulate pesticides in the US, the passage of FQPA assigned a newfound emphasis on the heightened vulnerability to pesticide exposure during childhood and on the cumulative health impacts of pesticide groups acting through common mechanisms of toxicity.[31] This placed enhanced scrutiny on the use of conventional pesticide groups including OP insecticides. As pesticide regulations changed in response to this scrutiny and in the face of mounting evidence of human health impacts, OP usage in the US decreased from 2000 to 2012 by 70%.[30] Even with this marked decrease, OP pesticide usage persists throughout the US.[32]

In the state of Washington, the use of many common OP pesticides such as chlorpyriphos continues to be reported as of 2017 especially in agricultural communities such as Yakima and Benton counties (Figure 2).[32,33] Usage of another OP, azinphos-methyl (AZM), has significantly decreased following a 2006 US EPA mandate to phase out AZM by 2012.[34,35] Although AZM holds historical importance for the management of the codling moth, a major pest impacting apple orchards in Washington state, the downward trajectory in its use is evident in both Yakima

and Benton counties as they transition to reduced risk insecticides and other alternative management strategies (Figure 2).[32,33,34] In addition to these long-term regulatory trends, there is evidence of fluctuations in OP pesticide exposure in the households by agricultural season that differs between households with and without a farmworker as presented in Figure 3.

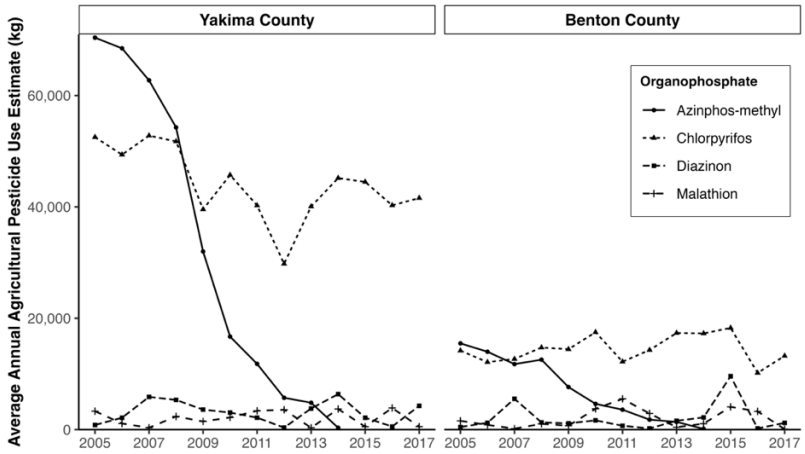

Figure 2. County-level average annual agricultural pesticide usage plotted from 2005-2017 using data derived from U.S. Geologic Survey.[32,33] In terms of kilograms used, azinphos-methyl is the primary OP pesticide reported for Yakima and Benton counties in 2005 and chlorpyriphos is the primary OP pesticide reported for 2011.

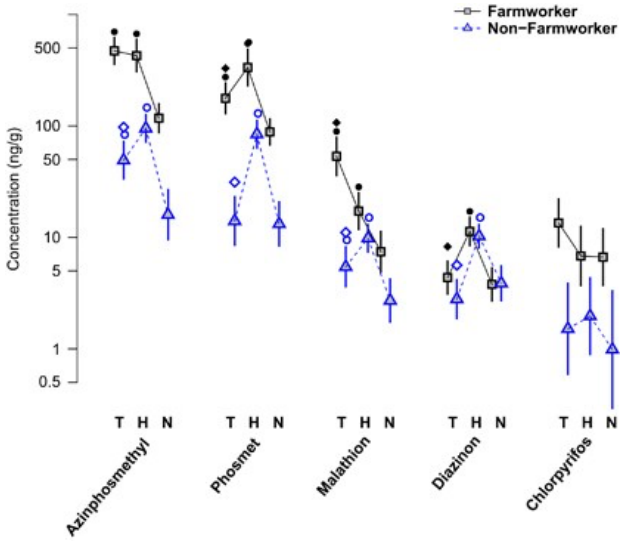

Figure 3. Plot of geometric means and 95% confidence limits of seasonal concentrations of OP pesticides detected in the house dust for families from the University of Washington's Center for Child Environmental Health Risks Research cohort living in Yakima and Benton counties in 2005. Figure 2 reprinted from Smith *et al.,* 2017.[36]

3.2 OP Pesticide Toxicity

Due to 1) widespread agricultural and at-home use, 2) the inherent production of dust and droplets during the application process, and 3) the persistence of residues on produce, people may be exposed to OPs through a variety of routes. For farmworkers, the predominant pathways are inhalation, non-dietary ingestion, and dermal absorption through direct occupational exposure during pesticide application, crop thinning, and crop harvest. For other members of agricultural communities, OP exposure has been found to stem from airborne pesticide drift from nearby farms, residues remaining on OP-sprayed produce, and, for families of farmworkers, para-occupational contact through take-home pathways.[37,38]

OPs are a diverse class of insecticides whose chemical composition is identified by a central phosphorous atom often covalently bonded to oxygen or sulfur.[39] The primary mechanism of acute toxicity for this class of pesticides is inhibition of acetylcholinesterase (AChE), an enzyme which facilitates the breakdown of the neurotransmitter acetylcholine. This inhibition of AChE results in the buildup of acetylcholine in cholinergic synapses and neuromuscular junctions.[8] The ensuing hypercholinergic activity can lead to muscle weakness, respiratory depression, seizures, coma, and death.[40] In addition to this acute neurotoxicity due to cholinergic overstimulation, an expanding body of evidence has begun to link low-level OP exposure to other adverse health impacts including asthma and type II diabetes mellitus through alternative mechanisms such as inflammation and oxidative stress. [6,8,41]

3.3 OP Pesticide Exposure Assessment

OP pesticide exposure can be characterized through questionnaires, environmental sampling, and biomarker measurement. Potential OP biomarkers include AChE inhibition in blood, parent compounds in blood, or metabolites in urine (Figure 4). In occupational settings with higher OP exposure, the measurement of AChE activity is frequently used since there is a dose-response relationship between exposure and inhibition, the analytical methods are easy to implement, and the results provide valuable insight into the toxicological health effects; however, this method does not have the sensitivity to assess exposure within the general population which may be below the assay's sensitivity or below the point of AChE inhibition.[42] On the other hand, while analyzing OP parent compounds allows for pesticide specificity, the analysis is more cumbersome and there are concerns over stability and rapid elimination. Considering these

limitations, measurement of OP metabolites in urine is an ideal biomarker of OP exposure for the general population as it is uniquely sensitive to low levels of exposures and collection of samples is non-invasive.[38,43]An example of the metabolic scheme of an OP pesticide, AZM, is shown in Figure 4 highlighting both specific and non-specific metabolites that result from its breakdown and can be measured in biological samples. Metabolites specific to AZM include mercaptomethyl benzazimide (MMBA) and hydroxymethyl benzazimide (HMBA), while non-specific urinary dialkylphosphate (DAP) metabolites include dimethyl phosphate (DMP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), diethylphosphate (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP). Each DAP metabolite is reflective of exposure to multiple OP parent compounds. Dimethyl phosphate metabolites (i.e. DMP, DMTP, and DMDTP) correspond to a group of dimethyl OPs including AZM and malathion while diethyl phosphate metabolites (i.e. DEP, DETP, DEDTP) correspond to a distinct group of diethyl OPs including chlorpyriphos and diazinon.[38]

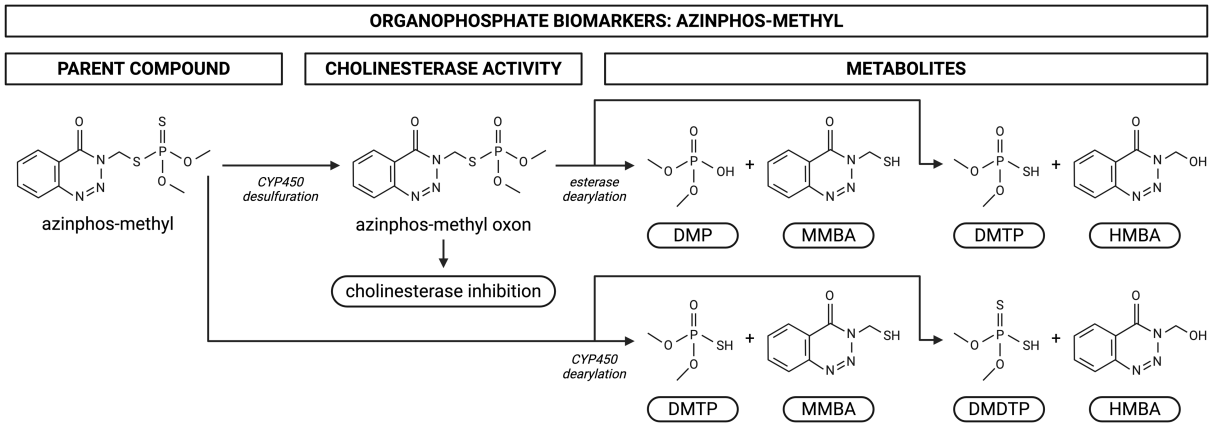

Figure 4. An example of a metabolic pathway for a dimethyl OP pesticide, AZM. Specific AZM metabolites highlighted here include HMBA and MMBA, while non-specific metabolites include DMP, DMTP, and DMDTP. These metabolites are excreted and can be measured in urine samples as a biomarker of OP exposure. Figure created with BioRender.com and adapted from Lin et al., 1980 and ATSDR, 2008.[44,45]

3.4 OP Pesticides and Respiratory Health

Considering epidemiological evidence, OP pesticides have been linked to respiratory symptoms and decreased lung function in various child and adult cohorts. [6,8,46] Shaffo *et al.* reports in their review that most meta-analyses as of 2018 suggest a strong association between OP exposure and asthma outcomes with one exception noting a weak association.[8] Although expanding in

recent years, the number of studies that focus on the respiratory health impact of OP exposure on children is currently limited. A study of a childhood asthma cohort from Baltimore City, Maryland found an association between specific markers of chlorpyriphos exposure and asthma morbidity; however, the findings were not significant or consistent across the other assessed OP pesticides (i.e. malathion, parathion, and diazinon).[47] In terms of respiratory symptoms in school-aged children, a paper focused on Northern Thailand found evidence of an association with DAP metabolites that was stronger for dimethyl than diethyl phosphate metabolites while another study in the Western Cape, South Africa found no consistent associations and advocated for repeated measures of urinary metabolites and extended follow-up in future studies.[48,49] A series of studies involving the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) birth cohort in the Salinas Valley, California illustrated a connection between levels of OP urinary metabolites and poor respiratory function test results as well as asthma symptoms in children between five and seven years old. [6,46]

3.5 OP Pesticides and Inflammation

Currently, in-vivo and in-vitro evidence of OP pesticide immunotoxicity points to inconsistent immunomodulatory effects. Most studies focus on the effects of diethyl insecticides such as parathion, diazinon, and chlorpyriphos that can be transformed via oxidative desulfurization into their oxon metabolite or hydrolyzed into non-specific diethyl phosphate metabolites.[50]

Animal studies using guinea pigs found that TNF-α release from activated macrophages can be induced by parathion exposure via subcutaneous injection and mediate airway hyperreactivity.[51] In a different study assessing allergic inflammation of the airway, pregnant mice were orally exposed to combinations of OP and organochlorine (OC) pesticides (i.e. parathion and methoxychlor) prior to induction of airway inflammation and subsequent immunotoxicity assessment of 8-week-old pups.[52] Increased infiltration of eosinophils in bronchoalveolar lavage fluid (BALF) and increases in IL-6 and IL-9 production in mice exposed to parathion were observed; however, this elevated cytokine production was only statistically significant for the group with combined exposure to parathion and methoxychlor. As for an in vitro model of differentiated human macrophages (THP-1 cells), parathion, diazinon, and chlorpyriphos parent compounds and DETP metabolites induced pro-inflammatory cytokine release in the form of TNF-α.[50] Similarly, other research utilizing murine in-vitro and in-vivo techniques found that

diazinon can both induce macrophage pro-inflammatory factors such as IL-6 and TNF-α while simultaneously inhibiting their phagocytic activity.[53]

Contrarily, another study highlighted immunosuppression of mast cells manifesting as decreased TNF-α and IL-4 release following stimulation with A23187 in the group treated with diazinon.[54] Watanabe *et al.* saw different markers of immunosuppression in the form of decreased IL-6 and IFN-γ levels in BALF when assessing the effects of perinatal methamidophos exposure in mice.[55] Unlike the OPs in the previously described studies, methamidophos does not break down into DEP, DETP, or DEDTP metabolites.

The epidemiological evidence of the association between OP pesticide exposure and plasma markers of inflammation is limited, especially in children. A recent study assessing the association between urinary DAP metabolites and a profile of cytokines in a cohort of male flower workers in Mexico found evidence of suppressed pro-inflammatory markers.[56] This was exhibited by increases in anti-inflammatory IL-10 as well as by decreases in IL-6 and the Th1 cytokines, IFN-γ and TNF-α. Th1 suppression of IFN-γ and TNF-α is stronger in dimethyl than diethyl metabolites while IL-6 suppression is stronger in diethyl than dimethyl metabolites.

ARTICLE

1. INTRODUCTION

Asthma threatens quality and, in severe cases, length of life for over 300 million people around the globe.[4] This chronic respiratory condition is a particularly pressing health concern for children (< 18 y) with the prevalence rate estimated at 6.2%, amounting to over 4.5 million cases in the United States alone.[1] Established risk factors associated with asthma pathogenesis include environmental exposures, family history of asthma, older age, male sex, and parental smoking.[5] While urban pollutants (e.g. vehicle exhaust) are well-established environmental determinants of asthma morbidity and mortality, emerging evidence suggests that other chemical exposures unique to agricultural communities (e.g. organophosphate (OP) pesticides) are also potential influencers of respiratory health. Specifically, epidemiological studies have linked early-life OP pesticide exposure to asthma outcomes and biomarkers in children, potentially through immunological pathways; however, mechanistic understanding of this relationship is limited.[47,46,6,49,48]

Cytokines have cultivated mounting interest in recent years for the molecular insight they are able to provide when strategically utilized in epidemiological studies. These molecules facilitate intercellular communication to coordinate immune and inflammatory responses in an effort to maintain homeostasis.[57] Cytokines' propensity to respond to an array of dynamic exposures such as OPs and their role in the pathogenesis of inflammatory diseases such as asthma makes them compelling biomarkers through which to consider both exposure and disease.[3] In terms of chronic OP exposure, epidemiological evidence highlights immunosuppression of Type 1 T-helper cell (Th1) cytokines which are implicated in cell-mediated immunity and protection against viral insults. [15,56] In vitro and in vivo evidence is more inconsistent with potential for variation due to the type of OP pesticide used in the model. Some studies indicate general induction of pro-inflammatory cytokines[50,51,52,53] while other studies highlight similar immunosuppression as is seen in humans.[54,55]

Historically, concerns with the inherent transience of cytokines throughout the body and a limited understanding of the complex, overlapping nature of their signaling pathways has posed challenges for interpretation of single analyte measurements in epidemiological studies. Fortunately, advancements in quantification techniques and a growing understanding of their unique, context-dependent functions has allowed for multiplexed measurement of more comprehensive cytokine arrays and addressed many of these concerns.[11] Considering this progress, measuring a profile of cytokine levels in biological media has become a powerful tool for characterizing unique patterns of inflammation for phenotypically diverse inflammatory conditions such as asthma.

The University of Washington Child Environmental Health Risks Research (CHC) cohort of children from both farmworker and non-farmworker families residing in an OP intensive agricultural region of Washington state provides an opportunity to investigate cytokine variability over time and across a population of children as well as explore relationships between low-level OP exposure and inflammatory responses related to respiratory health, notably asthma. We hypothesize that biomarkers of exposure and inflammation will be significantly different between agricultural seasons and demographic characteristics, higher OP exposure will be associated with suppression of pro-inflammatory cytokines, and higher OP exposure will be observed among children with an asthma diagnosis.

2. METHODS

2.1 Cohort Description

Throughout 2011, a longitudinal study of a subset of 100 households from the University of Washington Center for Child Environmental Health Risks Research (CHC) cohort was carried out. The larger CHC cohort has been previously described by Thompson *et al.* (2014).[37] The study population includes adult-child dyads split into 60 farmworker and 40 non-farmworker households. A dyad was considered part of a farmworker household if at least one family member worked on a farm for any of the three defined agricultural seasons in 2011 (i.e. pre-thinning, thinning, and non-spray). The pre-thinning season from March to May is characterized by the active application of OP pesticides such as chlorpyriphos, the thinning season from June to August involves trimming trees to promote fruit growth, and the non-spray season from November to December is a post-harvest period where pesticide application is reduced. These households are located in two counties, Benton and Yakima, in the Lower Yakima Valley of Washington that significantly contribute to the state's agricultural sector.

2.2 Sample Collection

2.2.1 Child Plasma Collection

A certified phlebotomist collected blood into a 10 mL lavender top collection tube from CHC children once per pre-thinning, thinning, and non-spray seasons in 2011. The tube was inverted, centrifuged, and the resulting EDTA plasma was aliquoted for storage in 4 mL cryovials. The cryovials were kept at -80˚C until biomarker analysis. Plasma samples across all three agricultural seasons were available for 67 of the 100 CHC children.

2.2.2 Child Urine Collection

Morning void urine samples were collected from CHC children on Day 1, 3, 5, and 7 of the pre-thinning, thinning, and non-spray seasons in 2011. With the exception of the pre-thinning season, not all urine samples collected at all four timepoints were analyzed. In the thinning season, urine collected on Day 1, 5, and 7 and in the non-spray season urine collected on Day 1 and 5 were analyzed. Urine samples were available for 98 of the 100 CHC children for a total of 390 samples analyzed for the pre-thinning season, 203 samples analyzed for the thinning season, and 189 samples analyzed for the non-spray season.

2.3 Laboratory Analysis

2.3.1 Cytokine Profile Measurement in Plasma

Concentrations of nine different cytokines (i.e. IL-1β, IL-4, IL-5, IL-6, IL-8, IL-17A, IFN-γ, MCP-1, and TNF-α) associated with respiratory outcomes were measured in all available child plasma samples utilizing a custom MesoScale Discovery multiplex sandwich immunoassay. Each sample was run in duplicate. The lower limits of detection were calculated as 2.5 standard deviations above the lowest point on the calibration curve and fell within the following ranges in the units of pg/mL across the plates used for the analysis: IL-1β [0.15, 0.19], IL-4 [0.03, 0.06], IL-5 [0.23, 0.28], IL-6 [0.24, 0.42], IL-8 [0.13,0.17], IL-17A [0.76, 0.90], IFN-γ [4.28, 5.22], MCP-1 [0.20, 0.26], and TNF-α [0.24, 0.29].

2.3.2 Dialkylphosphate (DAP) Metabolite Measurement in Urine

Using high performance liquid chromatography-linked tandem mass spectrometry (HPLC-MS/MS) as described by Odetokun *et al.*, dialkylphosphate (DAP) metabolites were measured in all available child urine samples as a non-specific biomarker of OP exposure.[58] Measurements were conducted for both dimethyl and diethyl phosphate metabolites and included dimethyl phosphate (DMP), dimethyl thiophosphate (DMTP), dimethyl dithiophosphate (DMDTP), diethyl phosphate (DEP), diethyl thiophosphate (DETP), dimethyl dithiophosphate (DEDTP). The levels of each metabolite in urine were reported without measurement of creatine. The lower limits of detection were 5, 2.5, 1, 5, 1, and 1 ng/mL, respectively.

2.4 Child Respiratory Health Assessment

During the non-spray season in 2011, a respiratory health questionnaire was administered to the parent or guardian within each adult-child dyad. These questionnaires were completed by 98 of the 100 recruited households and were used to determine both child and parental asthma status. The questionnaire was adapted from the International Study of Asthma and Allergies in Childhood (ISAAC) module for children aged 6-7. For this study, parental and child asthma status is defined by the response to the following questions from the distributed survey:

Has a doctor or other health professional ever told you that you have asthma?

Has a doctor or other health professional ever told you that your child has asthma?

2.5 Statistical Analysis

The following statistical analysis was completed in R Version 4.3.3, and the final code is found in the supplemental materials.

2.5.1 Bayesian Multivariate Modeling of Variability in Plasma Cytokine Levels by Agricultural Season and Child Demographics

To assess variability through analysis of fixed and random effects, each of the cytokines were modeled using multivariate log-normal models built via Bayesian Markov chain Monte Carlo (MC-MC) Hamiltonian sampling methods (Supplemental Equation 4). All the available cytokine concentration data from the subset of 67 households that provided child plasma samples was incorporated into the model. Cytokine concentrations are natural log transformed for modeling. This model accounts for fixed effects due to the agricultural season that the sample was collected, the child age, and the child sex. Individual child was accounted for as a random effect. Cytokine concentrations reported below the limits of detection were treated as left censored at the limit of detection. The detection rates for each cytokine are displayed in Table 4. Any cytokine that had a detection rate less than 15% was excluded from subsequent analyses.

The output of this multivariate model was evaluated to understand variability in the measurements for each cytokine. First, the conditional fixed effects were calculated to assess variability across agricultural season, age, and sex. Second, to understand between and within child variability in cytokine measurements, the variance of the random effects and the intraclass correlation coefficient (ICC), respectively, were calculated. The ICC index was calculated in R Version 4.3.3 using the *icc* function from the *performance* package.

2.5.2 Bayesian Multivariate Modeling of Variability in Urinary DAP Metabolites by Agricultural Season and Child Demographics

Using a similar method as described in Section 2.5.1, after conversion to nmol/mL, urinary concentrations of total dialkylphosphate (∑DAP), total dimethyl phosphate (∑DMP), and total diethyl phosphate (∑DEP) metabolites were estimated using multivariate log-normal models built via Bayesian Markov chain Monte Carlo (MC-MC) Hamiltonian sampling methods (Supplemental Equations 1-3). All the available urinary DAP metabolite concentration data from the subset of 98 households that provided urine samples was incorporated into the model. ∑DAP is calculated as the sum of DMP, DMTP, DMDTP, DEP, DETP, and DEDTP urinary metabolite

concentrations, ∑DMP is calculated as the sum of DMP, DMTP, and DMDTP urinary metabolite concentrations, and ∑DEP is calculated as the sum of DEP, DETP, and DEDTP urinary metabolite concentrations. ∑DAP, ∑DMP, and ∑DEP are natural log transformed for modeling. Data below the limits of detection were treated as left censored if all metabolites within the dose term fell under the limit of detection and interval censored if any metabolites within the dose term were above the limit of detection. As with the cytokine models described in Section 2.5.1, these models account for fixed effects due to the agricultural season that the sample was collected, the child age, and the child sex as well as random effects due to the individual child. The urinary DAPs were analyzed using the same effect terms as the plasma cytokines to facilitate subsequent analyses of the relationship between the urinary DAPs and plasma cytokines.

These multivariate models were evaluated to understand variability in the ∑DAP, ∑DMP, and ∑DEP. The conditional fixed effects were calculated to assess variability across agricultural season, age, and sex, and the variance of the random effects and the ICC were calculated to assess between and within child variability. Similar analyses assessing variability in urinary DAP metabolites have been previously published for other study years for this cohort.[59]

2.5.3 Linear Mixed Effects Regression of the Association Between ∑DAP, ∑*DMP and* ∑*DEP Urinary Metabolite Levels and Plasma Cytokine Levels*

The predictor variable, ∑DAP, ∑DMP, or ∑DEP urinary metabolite concentration, is a concentration in nmol/mL as estimated for each child for each agricultural season by the MC-MC modeled joint distribution. Similarly, the response variable, a cytokine concentration in pg/mL, is a concentration as estimated for each child for each agricultural season by their respective MC-MC modeled distributions. This analysis included a subset of 67 households that provided urine samples, plasma samples, and completed the respiratory health questionnaire. A linear mixed effect model is utilized to investigate the relationship between the concentration of urinary DAP metabolites and circulating levels of plasma cytokines with variability due to individual child accounted for as a random effect (Supplemental Equations 14-19). A mixed effect model is run for each MC-MC draw. To assess the relationship, the results of the series of regressions are used to calculate an average regression coefficient, a p-value, and a 95% confidence interval. For each of the cytokines, this process was completed for a base model as

well as for an adjusted model that controlled for sex, age, and the presence of a farmworker in the household as fixed effects. If the adjusted model was determined to be a significantly better fit via a likelihood ratio test, as indicated by a p-value of less than 0.05, then the adjusted model is reported in this analysis (see Supplemental Table 2). If not, then the base model will be reported. Additionally, effect modification due to the presence of a farmworker in the household, sex, and age is assessed through introducing interaction terms (Supplemental Equations 20-22). Interaction terms rather than stratification were chosen to assess effect modification due to the relatively small sample size of this cohort.[60]

2.5.4 Logistic Regression Analysis of the Association Between ∑DAP, ∑DMP and ∑DEP Urinary Metabolite Levels and Asthma Diagnoses

The predictor variable, ∑DAP, ∑DMP, or ∑DEP urinary metabolite concentration, is a concentration in nmol/mL averaged across all three agricultural seasons as estimated for each child by the MC-MC modeled joint distribution. The response variable is a binary outcome dependent on the presence or absence of an asthma diagnosis as determined by the administered respiratory health questionnaire. This analysis included a subset of 67 households that provided urine samples, plasma samples, and completed the respiratory health questionnaire. A logistic binomial regression is utilized to investigate the relationship between an individual's average concentration of urinary DAP metabolites throughout 2011 and their asthma status (Supplemental Equations 5-10). A regression is run for each MC-MC draw. To assess the relationship, the results of the series of regressions are used to calculate an average regression coefficient, p-value, and a 95% confidence interval. This process was completed for a base model as well as for an adjusted model that controlled for sex, age, presence of a farmworker in the household, parental asthma status, and the presence of a smoker in the household. Considering the available data for this cohort, these covariates were chosen based on literature evidence implicating them as potential confounders or risk factors for asthma development. The fit of the base and adjusted model were compared using a likelihood ratio test (see Supplemental Table 1). If the adjusted model was determined to be a significantly better fit, as indicated by a p-value of less than 0.05, then the adjusted model is reported in this analysis. If not, then the base model will be reported. Effect modification due to sex, age, the presence of a farmworker in the household, and parental asthma status was assessed through introducing interaction terms (Supplemental Equations 11-13, Supplemental Table 5).

3. RESULTS

3.1 Cohort Characteristics

Cohort characteristics for the 67 households that provided urine and plasma samples and completed respiratory health questionnaires are summarized in Table 3. The cohort is composed of 39 children with farmworkers in the household and 28 children without a farmworker in the household between 7 and 13 years old. The caregiver in these households primarily identify as of Hispanic/Latino origin. Overall, 19.4% of caregivers reported asthma in their child. This asthma burden is different depending on whether there is a farmworker present in the household with 23.1% of caregivers in households with a farmworker reporting an asthma diagnosis for their child compared with 14.3% in non-farmworker households; however, there is a higher proportion of female children in the non-farmworker group which may contribute to this discrepancy.

Table 3. Adult and child characteristics, 2011 University of Washington Center for Child Environmental Health Risks Research (CHC) cohort, n=67

		Parental Occupation Status	
Parent/Guardian is the Respondent	**Overall**, N = 67[1]	**Farmworker**, N = 39[1]	**Non-farmworker**, N = 28[1]
Do you consider yourself of Hispanic/Latino origin?			
Yes	66 (98.5%)	39 (100.0%)	27 (96.4%)
No	1 (1.5%)	0 (0.0%)	1 (3.6%)
Child's Age (years)			
7 to 9 years old	21 (31.3%)	13 (33.3%)	8 (28.6%)
10 to 11 years old	29 (43.3%)	15 (38.5%)	14 (50.0%)
12 to 13 years old	17 (25.4%)	11 (28.2%)	6 (21.4%)
Child's Sex			
Female	37 (55.2%)	20 (51.3%)	17 (60.7%)
Male	30 (44.8%)	19 (48.7%)	11 (39.3%)
Has a doctor or other health professional ever told you that your child has asthma?			
Yes	13 (19.4%)	9 (23.1%)	4 (14.3%)
No	54 (80.6%)	30 (76.9%)	24 (85.7%)
Has a doctor or other health professional ever told you that you have asthma?			
Yes	5 (7.5%)	3 (7.7%)	2 (7.1%)
No	62 (92.5%)	36 (92.3%)	26 (92.9%)
Does anyone in your household smoke inside the house?			
Yes	1 (1.5%)	1 (2.6%)	0 (0.0%)
No	66 (98.5%)	38 (97.4%)	28 (100.0%)

[1]n (%)

3.2 Urinary DAP Metabolite & Plasma Cytokine Levels for CHC Children in 2011

∑DMP was generally at a higher level and had more datapoints above their limits of detection than ∑DEP in child urine samples. As for the measured cytokine profile, the analytes with the most data points above the limit of detection were IFN-γ and MCP-1 reporting 100% detection rates in children's plasma. The number of datapoints above the limit of detection for IL-4 was much lower at around a 3% detection rate, so IL-4 was excluded from further analysis. The 2011 urinary DAP metabolite and plasma cytokine levels for the CHC children are summarized in Table 4 as estimated by the joint distributions of Bayesian mixed effects models fit via MC-MC Hamiltonian sampling (see Supplemental Equations 1-3). The parameters defining these models can be found in Supplemental Table 3 and 4.

Table 4. Summary statistics for DAP metabolite and cytokine levels in urine and plasma samples collected throughout 2011 as estimated by the joint distributions of Bayesian mixed effects models fit via MC-MC Hamiltonian sampling methods. Values below LOD were treated as left or interval censored for the urinary DAP metabolites and left censored for the plasma cytokines.

				Percentiles[1,2]				
Analyte	**n**	**%>LOD**	**mean**	**10th**	**25th**	**50th**	**75th**	**90th**
∑DAP[1]	764	21%	0.119	0.060	0.071	0.086	0.105	0.127
∑DMP[1]	764	27%	0.074	0.037	0.042	0.049	0.056	0.064
∑DEP[1]	764	16%	0.036	0.018	0.022	0.027	0.033	0.041
IL-1β[2]	201	55%	0.898	0.167	0.255	0.409	0.674	1.098
IL-4[2,3]	201	3%	-	-	-	-	-	-
IL-5[2]	201	47%	0.482	0.131	0.200	0.329	0.505	0.720
IL-6[2]	201	80%	0.743	0.324	0.424	0.565	0.768	1.113
IL-8[2]	201	88%	5.537	2.254	3.037	4.211	5.831	7.796
IL-17[2]	201	81%	1.418	0.644	0.826	1.081	1.431	2.050
IFN-γ[2]	201	100%	17.348	7.474	9.526	12.554	16.831	22.470
MCP-1[2]	201	100%	148.056	86.031	107.105	137.194	165.876	199.678
TNF-α[2]	201	93%	1.053	0.611	0.717	0.875	1.133	1.570

[1]Percentiles reported in nmol/mL of urine, [2]Percentiles reported in pg/mL of plasma, [3]IL-4 was excluded from estimation via Bayesian mixed effects MC-MC methods

3.3 Results of Bayesian Multivariate Models Assessing Plasma Cytokine Variability

The conditional fixed effects and 95% credible intervals by agricultural season as well as by the interaction between sex and age as estimated by the Bayesian mixed effects models fit via MC-MC sampling methods are displayed in Figure 5. The complete results of the Bayesian

multivariate model can be found in Supplemental Table 4. Generally higher values were observed for IFN-γ, TNF-α, IL-1β, IL-6, IL-17A, and IL-8 during the pre-thinning season and MCP-1 and IL-5 during the thinning season. Out of the eight analyzed cytokines, statistically significant seasonal variation, as determined by an absence of overlapping credible intervals was seen for IFN-γ and IL-8. For IFN-γ, this manifested as a significant decrease from the pre-thinning to thinning season and from the pre-thinning to non-spray season, while for IL-8 there was a significant decrease from the pre-thinning to non-spray season. In terms of demographic variability, while cytokine levels trended higher for males, considering the overlapping confidence intervals across age ranges and between sexes, no significant variation was indicated (Figure 5).

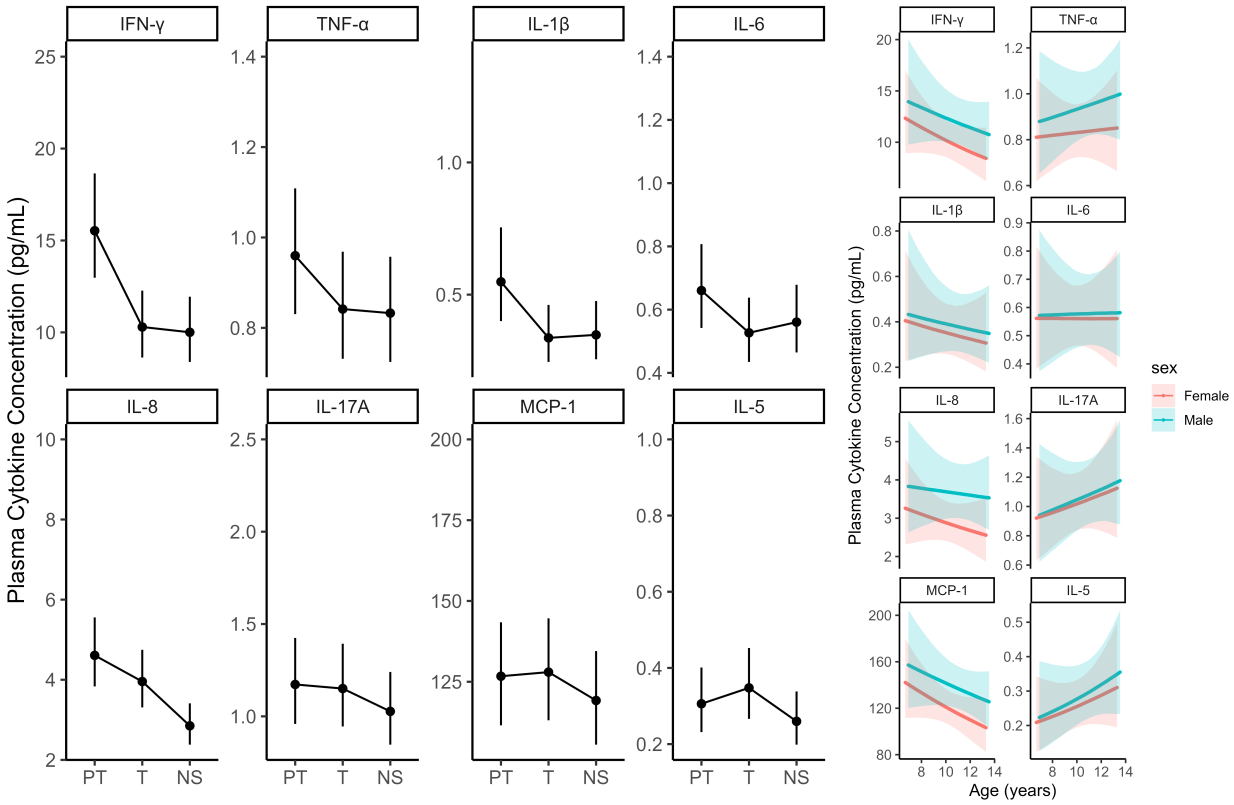

Figure 5. Conditional fixed effect estimates with error bars representing the 95% credible interval for plasma cytokine levels by agricultural season and by the interaction between sex and age. These estimates were modeled using Bayesian mixed effects methods fit via MC-MC Hamiltonian sampling (Supplemental Equations 1-3). Values below the LOD were treated as left censored for cytokines.

PT: pre-thinning season, T: thinning season, NS: non-spray season

In addition to the fixed effects outlined above, modeling the random effects on cytokine levels due to individual child using these Bayesian MC-MC models provides a basis through which to assess within and between child variability. As seen in Figure 6, visually, within child variability tends to increase as the plasma cytokine level increases. Statistically, the within child variability is represented by the ICC with lower ICC values indicating greater variability for within a group, and the between child variability is represented by the variance of the random effects (σ^2) with higher values indicating greater between child variability. The greatest within child variability, as indicated by a relatively low ICC of 0.18, is observed for IFN-γ while the greatest between child variability is observed for IL-1β with a random effects variance of 0.52 (Figure 6).

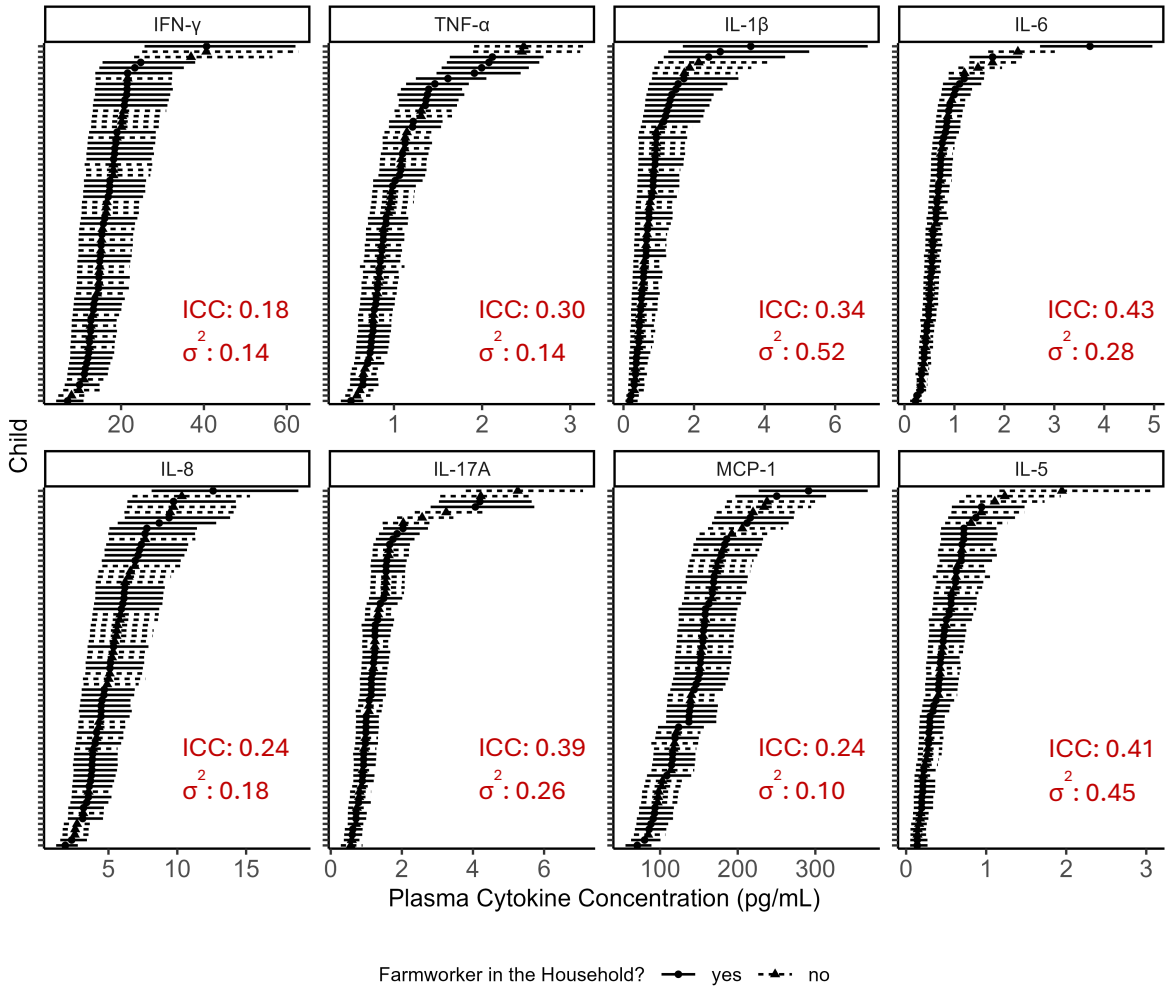

Figure 6. Random effects and 95% credible intervals for plasma cytokine levels for each child within the CHC cohort in 2011, as estimated by the Bayesian mixed effects models fit via MC-MC Hamiltonian sampling methods (Supplemental Equations 1-3)

ICC: intraclass correlation coefficient, σ^2: variance of random effects

3.4 Results of Bayesian Multivariate Models Assessing Urinary DAP Variability

As estimated by the Bayesian mixed effects models fit via MC-MC sampling methods, the conditional fixed effects and 95% credible intervals by agricultural season as well as by the interaction between sex and age are displayed in Figure 7. The complete results of the Bayesian multivariate model can be found in Supplemental Table 3. Generally higher values of urinary DAPs were observed during the thinning season; however, considering the similar credible intervals between seasons for all three analytes, this trend is not significant. Similarly, as indicated by the overlapping credible intervals across age ranges and between sexes, no significant demographic variation was indicated for this population of children.

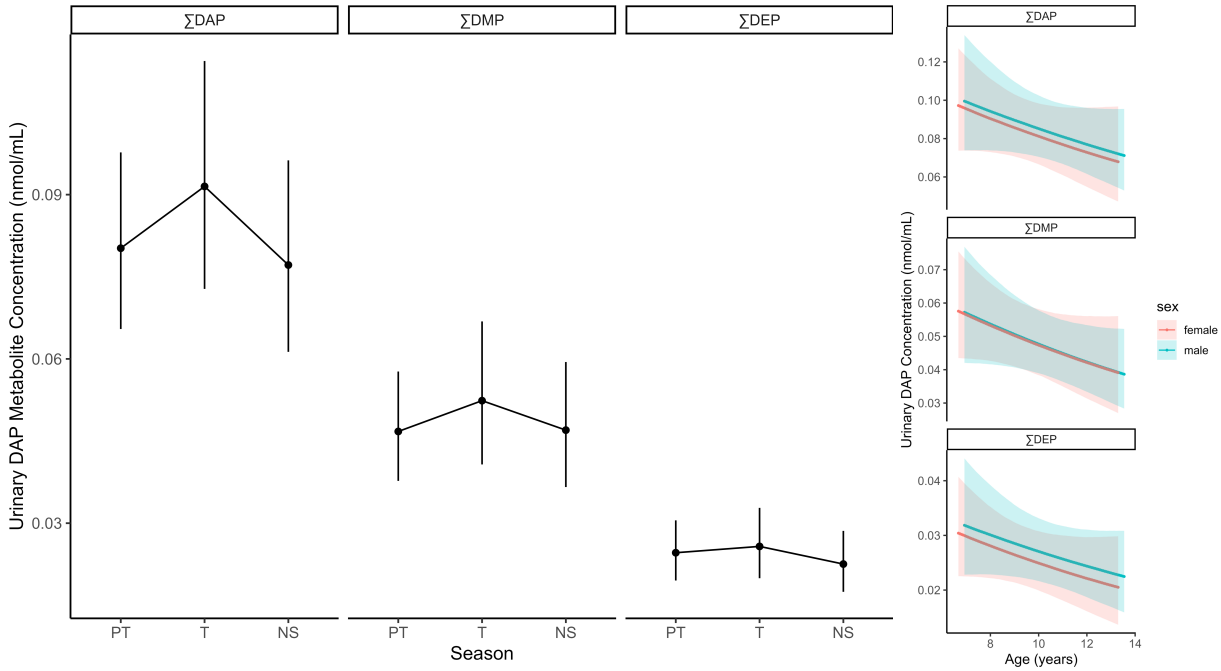

Figure 7. Conditional fixed effect estimates with error bars representing the 95% credible interval for urinary DAP metabolites for each agricultural season in 2011 as well as for the interaction between sex and age. These estimates were modeled using Bayesian mixed effects methods fit via MC-MC Hamiltonian sampling (Supplemental Equations 1-3). Values below the LOD were treated as left or interval censored.
PT: pre-thinning season, T: thinning season, NS: non-spray season

The random effects estimated for urinary DAP levels by individual child are represented in Figure 8. Notably, the greatest within child variability is observed for ∑DMP, as indicated by a relatively low ICC of 0.02, while a random effects variance of 0.10 suggests the greatest between child variability for ∑DEP (Figure 8).

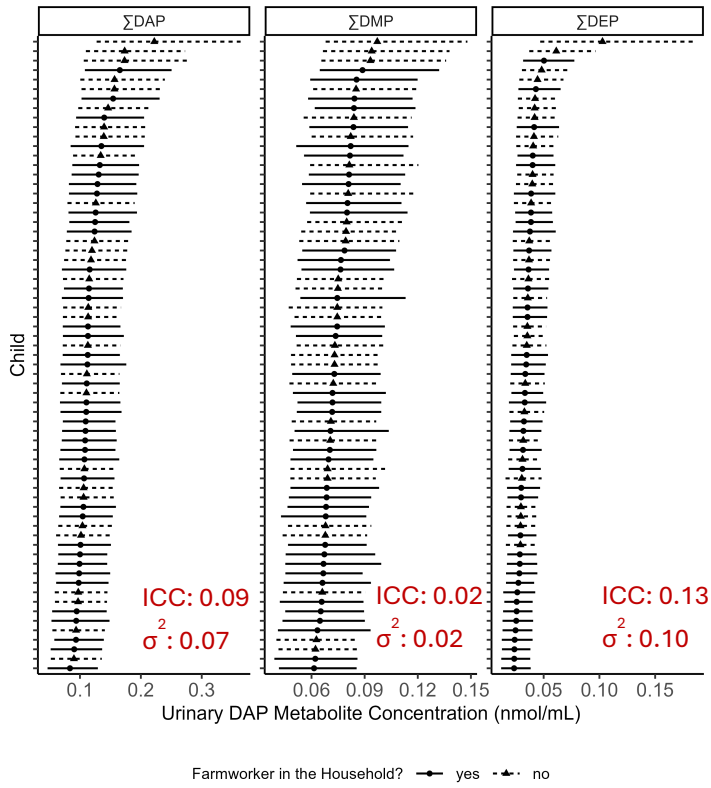

Figure 8. Random effects and 95% credible intervals for urinary DAP metabolite levels for each child within the CHC cohort in 2011, as estimated by the random effects of the Bayesian mixed effects models fit via MC-MC Hamiltonian sampling methods (Supplemental Equations 1-3).

ICC: intraclass correlation coefficient, σ^2: variance of random effects

3.5 Association Between Urinary DAP Metabolites and Plasma Cytokine Levels

As estimated by linear mixed effects regressions, the associations between urinary DAP metabolite concentrations and the measured profile of circulating cytokines after adjusting for the presence of a farmworker in the household, sex, and age are displayed in Figure 9. The adjusted models are reported here as they represent a significantly better fit with p-values less than 0.05 (see Supplemental Table 2). No significant relationships between urinary DAP metabolites and cytokine levels are noted using this method; however, considering the average trends, the strongest relationships ($p < 0.2$) observed are negative relationships between ∑DAP levels and TNF-α and IL-6 levels as well as a positive relationship between ∑DAP and IL-5 (Figure 9).

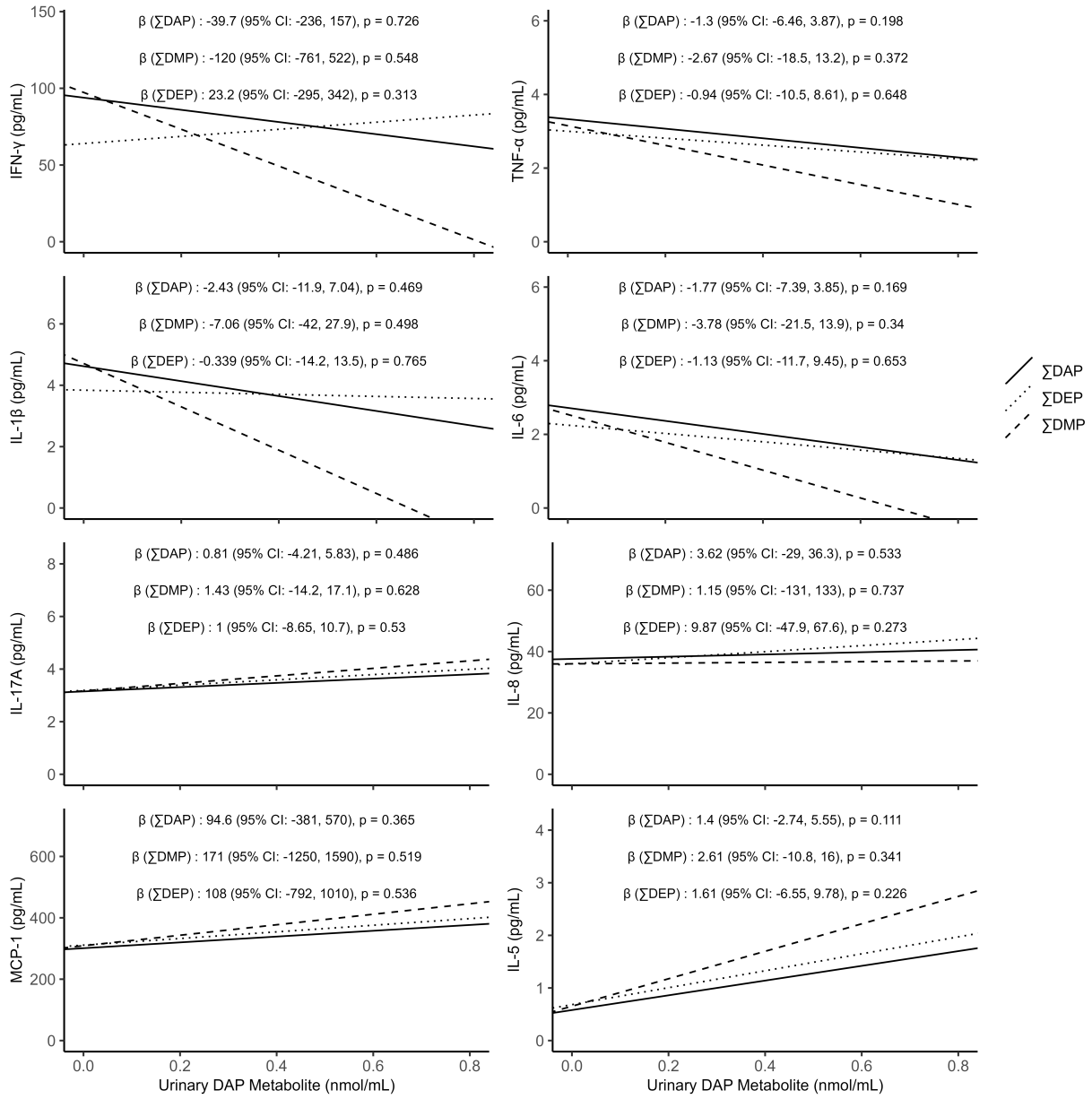

Figure 9. Associations determined via linear mixed effects regressions between urinary DAP metabolites (i.e. ∑DMP and ∑DMP) concentrations and plasma cytokine (i.e. IFN-γ, TNF-α, IL-1β, IL-6, IL-17A, IL-8, MCP-1, and IL-5) concentrations for children in living in the Lower Yakima Valley in 2011. Regressions were adjusted for the presence of a farmworker in the household, child sex, and child age.

No significant effect modification was observed due to season of sample collection, the presence of a farmworker in the household, child sex, or child age, as indicated by $p > 0.1$ for all interaction terms (see Supplementary Table 3).

3.6 Association Between Urinary DAP Metabolites and Child Respiratory Health

A visual representation of the results of the logistic binomial regression of average ∑DAP, ∑DMP, and ∑DEP over the 2011 study period as estimated by the joint distribution of Bayesian mixed effects models fit via MC-MC Hamiltonian sampling methods on the presence of an asthma diagnosis is presented in Figure 10. The model controlling for the child's sex, the child's age, the presence of a farmworker in the household, a reported parental history of an asthma diagnosis, and the presence of a smoker in the household was not a significantly better fit as indicated by p-values greater than 0.05, so the base model is reported here. The results of the regression do not support an association between average urinary DAP levels and the probability of having an asthma diagnosis for ∑DAP (β: -4.08 (95% CI: -20.6, 12.5), p = 0.598), ∑DMP (β: -6.32 (95% CI: -46, 33.4), p = 0.694), or ∑DEP (β: -18.8 (95% CI: -52.8, 25.2), p = 0.342). No significant effect modification was observed due to child sex, child age, the presence of a farmworker in the household, or parental history of an asthma diagnosis, as indicated by $p > 0.05$ for all interaction terms (Supplemental Table 5).

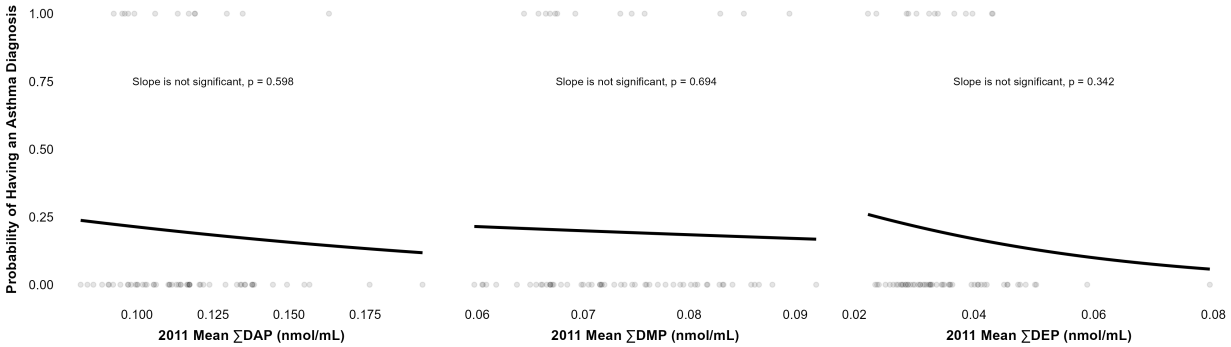

Figure 10. Logistic binomial regressions highlighting a non-significant decrease in the probability of having an asthma diagnosis as average urinary DAP metabolite level increases for children living in the Lower Yakima Valley in 2011.

4. DISCUSSION

The objective of this analysis was to assess cytokine variability over time and across a population of children as well as explore relationships between low-level OP exposure and inflammatory responses related to respiratory health, notably asthma. In general, significant seasonal variability was found for levels of IFN-γ and IL-8 in child plasma for this agricultural cohort; however, no significant associations were discerned between OP exposure,

inflammation, and asthma. To our knowledge, this is the first child cohort study assessing the relationship between individual markers of OP pesticide exposure and circulating cytokine levels.

4.1 Asthma Prevalence in the Lower Yakima Valley CHC Cohort

The total child asthma prevalence for this cohort is reported at around 19.4% (Table 3). These prevalences exceed the 2011 United States National Health Interview Survey prevalence for children between the ages of 5 and 14 and the Washington Behavioral Risk Factor Surveillance System prevalence for children between the ages of 10 and 14 estimated at 10.6% and 9.2%, respectively.[61,62] Even though the small sample size limits generalizability of these findings, there are clear elevations in asthma prevalence compared to the general Washington and United States population. To our knowledge, this study is the first to investigate OP pesticides as a driver of these disparate child respiratory health outcomes in this Washington agricultural community and provides unique insight into potential immunological mechanisms at play.

4.2 Seasonal and Demographic Variability

4.2.1 Variability in Plasma Cytokine Levels

Although no significant variation in circulating cytokine levels was observed across sex or age, significant variation across agricultural seasons was observed for the pro-inflammatory Th1 cytokine, IFN-γ, and the neutrophilic cytokine, IL-8. For both of these cytokines, levels were significantly lower in the fall during the non-spray season than when the first samples were taken in the spring during the pre-thinning season (Figure 5).

It is understood that, even in healthy individuals, cytokine levels vary temporally due to changes in environmental factors such as climate and allergens over the year. However, there is a lack of evidence addressing whether seasonal changes in pesticide exposure unique to agricultural communities will have a substantial impact on these trends. There is also a gap in the understanding of these seasonal cytokine trends during early-life periods.

A study of healthy adults conducted by Ter Horst *et al.* under the Human Functional Genomics Project found a peak in pro-inflammatory cytokine production during the summer.[63] Similarly, in a healthy, urban cohort of adults, Myrianthefs *et al.* described the cytokine response as primarily pro-inflammatory in early summer, a phenotype which was seen to be suppressed upon the

arrival of autumn.[64] The setting of this urban cohort is distinct from that of the rural, agricultural CHC cohort in the Lower Yakima Valley, and the seasonal cytokine trends are equally as distinctive. In fact, for the 2011 CHC cohort, some of the lowest estimated mean levels of pro-inflammatory cytokines (i.e. TNF-α, IL-1β, IL-6) were observed during the thinning season, which is reflective of early summer for the children in the Lower Yakima Valley (Figure 5). At this time, there is only one study to our knowledge characterizing seasonal changes in cytokine levels for a cohort including children which, unlike the CHC cohort, saw no seasonal variability for IL-8 or IFNγ-induced protein (IP)-10.[65] Considering the study settings, it is possible that an exposure, like OP pesticides, uniquely high during the thinning season for children in agricultural communities may be a driver of the dichotomy of immune signaling between people living in urban and rural areas. This would align with the evidence of OP-induced immunosuppression observed in other epidemiology studies.[15,56] There are also other key differences such as age of the study populations that could be driving this incongruity in seasonal trends between cohorts. It is possible the differences in trends between adult and child studies could be reflective of changes in the immune system that occur during development. While the spring and summer trends were different across studies, the CHC cohort did see suppression of cytokine levels during the fall (see Figure 5) similar to what was observed by the urban cohort described by Myrianthefs *et al*.[64] Further investigation is warranted to fully understand the similarities and differences in seasonal cytokine trends between regions and life stages.

As for the described random effects of individual child on cytokine levels, this is the first study to our knowledge that has assessed within and between child variability for cytokines in this manner (Figure 6). This intricate understanding of trends within individuals and across populations is important to support the appropriate development and allocation of public health resources and interventions addressing inflammatory conditions. Moreover, the ability to produce these Bayesian MC-MC models, parse out this child variability, and use the resulting distributions for subsequent regressions will ensure that uncertainty is not underestimated when combining datasets to model exposure response relationships.

4.2.2 Variability in Urinary DAP Metabolites

Urinary DAP metabolites have been well-characterized for this cohort for previous study years in earlier publications where further discussion of variability in urinary DAP levels for this

community is found.[59,66] For the 2011 CHC study period discussed here, no significant variability by season, sex, or age was observed for urinary DAP levels (Figure 7).

It is important to note that the urinary DAP metabolites measured in this study are considered non-specific biomarkers of exposure since each metabolite may be reflective of exposure to multiple OP parent compounds. The ∑DMP dose term includes the dimethyl phosphate metabolites DMP, DMTP, and DMDTP which correspond to a group of OPs including AZM and malathion. As for the ∑DEP dose term, DEP, DETP, and DEDTP are metabolites of a distinct group of OPs including chlorpyriphos and diazinon.[38] In earlier work with the CHC cohort, Smith *et al.* and Thompson *et al.* reported seasonal trends in these OP parent compounds (i.e. AZM, malathion, chlorpyriphos, and diazinon) in household dust as well as seasonal trends in urinary metabolites (i.e. DMP, DMTP, DMDTP, DEP, DETP, DEDTP) for children living in the Lower Yakima Valley in 2005.[36,37] These studies help validate this method of exposure characterization through illustrating consistent trends across biological and environmental media. They also highlighted a decrease in dimethyl and diethyl parent compounds in house dust and dimethyl urinary metabolites from the thinning season to the non-spray season in 2005. As illustrated by Figure 7, although non-significant, this trend from thinning to non-spray season was mirrored in the urinary biomarkers of OP exposure measured for the 2011 study period. Notably, this seasonal trend in dimethyl urinary metabolites is consistent between 2005 and 2011 despite drastic changes to the OP pesticide landscape in Yakima and Benton counties with the phase out of azinphos-methyl mandated by the EPA in 2006.[34,35]

Generally, the mean ∑DMP and ∑DEP concentrations in child urine for the CHC children are 0.074 nmol/mL and 0.036 nmol/mL as estimated by Bayesian mixed effects models fit via MC-MC methods (Table 4). For comparability to other study populations, the geometric mean and 95% confidence interval for ∑DMP and ∑DEP concentrations in child urine for the CHC children in 2011 has also been calculated through imputing any value below limit of detection as the limit of detection divided by the square root of two. Using this method, the geometric mean ∑DMP and ∑DEP concentrations are estimated at 0.083 nmol/mL and 0.045 nmol/mL, respectively, for the 7 to 13 year old CHC children living in the Lower Yakima Valley in 2011. Regardless of methodology used, the mean urinary ∑DMP and ∑DEP concentrations for the CHC children are higher than the mean urinary ∑DMP (0.038 nmol/mL) and ∑DEP (0.020

nmol/mL) concentrations for the 6 to 11 year olds participating in the US National Health and Nutrition Examination Survey (NHANES) survey that same year. These elevated biomarker levels are reflective of higher OP exposure for this population of children compared to the general United States population (Supplementary Table 4).

4.3 OP-induced Respiratory-Associated Immunotoxicity

When relating OP-induced immunomodulation to respiratory health, previous studies have leveraged a variety of frameworks to tease out the role of individual cytokines within immune and inflammatory signaling pathways.[3,15,28] These frameworks characterize cytokines either by their function or by the source and/or target of their signaling. Three primary frameworks utilized in terms of child asthma are 1) comparing levels of signaling through pro- and anti-inflammatory pathways, 2) assessing the balance between Th1, Th2, and Th17 cell signaling, and 3) characterizing neutrophilic versus eosinophilic activity.

4.3.1 Pro-inflammatory and Anti-inflammatory Signaling Pathways

Within the measured profile, IFN-γ, TNF-α, IL-1β, and IL-6 are considered pro-inflammatory cytokines while IL-4 is more characteristic of anti-inflammatory responses. IL-4 was excluded from this study due to the considerably low detection rate at only 3% above the limit of detection. Pro-inflammatory cytokine responses in response to pathogenic insults, pollution, and allergens is considered a protective mechanism; however, chronic cytokine signaling and inflammation in the airway has the potential to damage epithelial cells, influence airway remodeling, and have consequences for lung function. In epidemiological applications, immunosuppression of pro-inflammatory cytokines (IFN-γ, IL-6, and TNF-α) was found to be associated with urinary OP metabolites in an adult cohort of male flower workers in Mexico.[56] Similarly, while not significant, the strongest associations seen for the CHC cohort was the suppression of pro-inflammatory cytokines (i.e. TNF-α and IL-6) with increasing urinary $\sum$DAP (Figure 9).

In vitro and in vivo studies exploring potential mechanisms of acetylcholinesterase-independent toxicity have observed more inconsistent results with some models indicating immunosuppression and other models pointing to a potentially upregulated pro-inflammatory response.[67,50,54] Guinea pig, mouse, and differentiated human THP-1 models assessing the effect of parathion, diazinon, and chlorpyriphos, and diethyl phosphate metabolite exposure highlight

airway hyperreactivity, the release of IL-6 and TNF-α from activated macrophages, and upregulation of IL-6 in hilar lymph nodes.[53,51,50,52] This contrasts with the pro-inflammatory suppression seen in a mouse model assessing the effects of perinatal methamidophos exposure.[55] It is notable that unlike chlorpyriphos and diazinon from the previous studies, methamidophos is not hydrolyzed into diethyl metabolites. This variation and the variation within the CHC cohort observed between ∑DMP and ∑DEP for IFN-γ, may be attributed to the potential for different OP parent compounds and their metabolites to have unequal and mechanistically distinct effects (Figure 9).[67]

4.3.2 Neutrophilic and Eosinophilic Signaling Pathways

Another method of characterizing asthma phenotypes is through assessment of whether neutrophilic or eosinophilic signaling dominates, with neutrophilia often associated with heightened symptom severity.[3] IL-8 secreted by airway epithelial cells is a chemokine involved in recruiting neutrophils to induce airway inflammation. Similarly, IL-17A is secreted from a variety of sources (e.g. Th17 cells) and recruits neutrophils indirectly through stimulating IL-8 release from epithelial cells and directly through acting on the neutrophils themselves. While not reported to act directly on neutrophils, IL-6 supports this neutrophilic activity through playing a key role in Th17 differentiation.[3] On the other hand, inflammation mediated through eosinophils is primarily promoted through Th2 pathways and is often associated with allergic responses such as those mediated through IL-5 signaling.[3] Following secretion from Th2 cells, IL-5 plays a key role in eosinophil and has been associated with asthma and atopy in a longitudinal birth cohort study.[20] Similar, another cytokine, MCP-1 is known to be secreted from airway epithelial cells and facilitate hyper-eosinophilic syndromes.[25]

For the CHC cohort, while non-significant, stronger relationships were observed for eosinophil associated cytokines (i.e. IL-5) in response to OP exposure manifesting as elevated levels in response to increasing ∑DAP concentrations (Figure 9).

4.3.3 Th1, Th2, and Th17 Signaling Pathways

Th1 responses, often characterized by the cytokines IFN-γ and TNF-α, are drivers of cell-mediated immunity and phagocytic functions protective against pathogenic insults. This differs from Th2 responses which are defined by IL-4 and IL5 release. Th2 signaling facilitates atopic and eosinophilic activity while suppressing certain protective responses to pathogens through the

production of anti-inflammatory cytokines.[68] Historically, the balance between Th1 and Th2 responses have been the primary focus child asthma epidemiology studies; however, the role of Th17 cells in respiratory health has been increasingly described in recent years. Th17 responses associated with IL-6 and IL-17A signaling have been seen to disrupt immunological tolerance which can often lead to chronic pro-inflammatory phenotypes consistent with disease like asthma.[69]

A study in the Western Cape province of South Africa assessed the relationship between OP pesticide exposures, T-helper cell cytokine responses, and asthma outcomes for a cohort of women in rural communities. Significant positive associations between ∑DAP and Th2 cytokine, IL-5, as well as between ∑DAP and Th17 cytokine, IL-17, were found; however, there were no significant associations between DAP metabolites and asthma status. The Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) birth cohort found associations between OP urinary metabolites and asthma as well as increases in the ratio of Th2 to Th1 associated cytokines for children between five and seven years old with a mother who is a farmworker.[46,6,15] This elevated Th2 response and suppressed Th1 response was signified by increasing IL-4 and decreasing IFN-γ levels.[15] IL-4 is thought to induce synthesis of IgE antibodies leading to airway hyperresponsiveness and excess mucus production while IFN-γ is thought to play a more protective role against asthma exacerbations, so this imbalance toward Th2 responses with early life exposure to an agricultural environment aligns as expected with their findings of decreased respiratory function and increased asthma outcomes associated with OP exposure.[6,15]

In the CHC cohort, the inability to include IL-4 limited assessment of the traditional Th2/Th1 ratio (i.e. IL-4/IFN-γ); however, using IL-5 as an alternative cytokine representative of Th2 responses demonstrates a Th2/Th1 imbalance toward Th2 signaling with increasing ∑DAP exposure for the CHC cohort. Specifically, non-significant elevated levels of Th2 cytokine, IL-5, are observed in response to higher urinary ∑DAP levels for the CHC cohort in the Lower Yakima Valley (Figure 8). The described epidemiological studies assessing OP exposure via urinary DAP metabolites and inflammatory responses via cytokine levels are compared to the CHC cohort and summarized in Table 5.

Table 5. Epidemiological studies assessing the relationship between urinary DAP metabolites and immune and inflammatory signaling

	Study Year	Location	Age (years)	Median (IQR) ∑DAP	Findings
Male Flower Workers in Mexico	2004	Morelos & the State of Mexico	Mean: 33	1.47 (0.78, 3.69) μmol/g creatinine	Positive associations between ∑DAP & IL-5 Negative associations between ∑DAP & IFN-γ, ∑DAP & TNF-α, and ∑DAP & IL-6
Rural Women Workers in South Africa	2009	Western Cape, South Africa	Median: 33	133.59 (41.86, 229.09) μg/g creatinine	Positive associations between ∑DAP & IL-5 and ∑DAP & IL-17
CHC	2011	Lower Yakima Valley, WA	Median: 10	0.086 (0.071, 0.105) μmol/mL urine	Non-significant positive associations between ∑DAP & IL-5 Non-significant negative associations between ∑DAP & TNF-α and ∑DAP & IL-6

4.3.4 Cohesive Assessment of Signaling Frameworks

Understanding where individual cytokines fall within the discussed frameworks and how these frameworks overlap is essential to appropriately interpret these complex, context-dependent trends in circulating cytokine levels. Figure 11 visually outlines the complexity of the cytokine signaling network discussed throughout this paper and highlights the average trends of the linear mixed regressions of cytokine levels on ∑DAP metabolite levels. As one would expect, the cytokine response to OP pesticide exposure appears to be aptly similar within groups of cytokines with comparable functional properties. IL-6 is a prime example of the overlapping, context-dependent nature of cytokines. It has been shown to play a role within many facets of this respiratory framework; Here, in the context of the 2011 CHC cohort, the IL-6 response to OP exposure seems to mirror that of the other pro-inflammatory cytokines rather than that of the other Th17 cytokines.

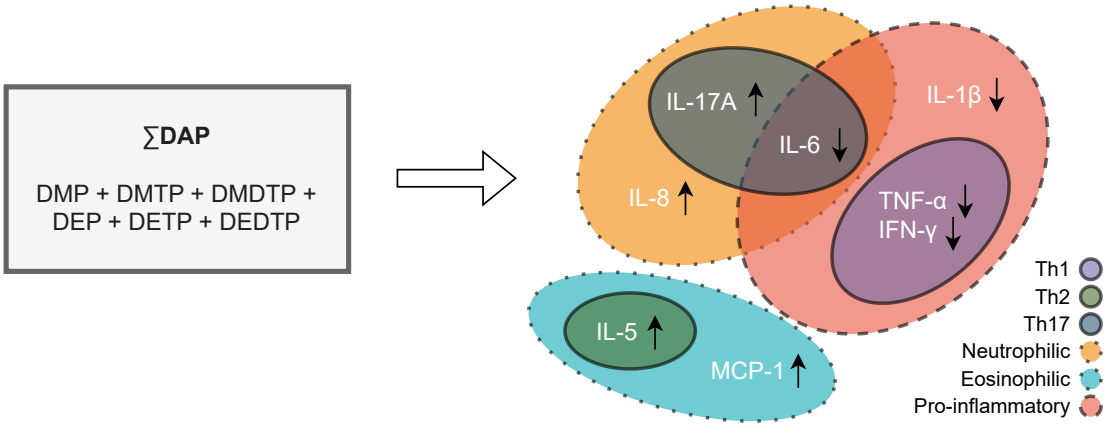

Figure 11. Visual summary of the direction of the average linear mixed effects regression coefficients of the association between urinary ∑DAP metabolites and circulating plasma cytokine levels. The cytokines are grouped by their respective, overlapping functional signaling groups. Positive and negative relationships are indicated by upward and downward arrows, respectively. As discussed in the results, none of these relationships were found to be statistically significant, so these arrows indicate generally which direction the association trends based off the mean regression coefficient.

4.4 Association Between Urinary DAP Metabolites and Child Respiratory Health

For the CHC children in the Lower Yakima Valley, no association was observed between average OP exposure across the 2011 study period and having a caregiver reported asthma diagnosis (Figure 10). The number of studies that focus on the respiratory health impact of OP exposure on children continue have expanded in recent years, with a high number of non-significant findings like those of the CHC cohort.

A series of studies involving the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) birth cohort in California did illustrate a significant connection between higher levels of OP urinary metabolites and poor respiratory function test results as well as asthma symptoms in children between five and seven years old. [6,46] A study of a childhood asthma cohort from Baltimore City, Maryland found an association between specific markers of chlorpyriphos exposure and asthma morbidity; however, the findings were not significant. [47] Another study in the Western Cape found no consistent associations between urinary DAP and respiratory symptoms in school-aged children, so they advocated for repeated measures of urinary metabolites and extended follow-up in future studies. [49] The Lower Yakima Valley CHC cohort successfully addresses these limitations with measurements of urinary metabolites on

multiple days over multiple seasons. One last paper focused on school-children in Northern Thailand found evidence of an association between DAP metabolite levels in urine and recent respiratory symptoms that was stronger for dimethyl than diethyl phosphate metabolites, despite generally lower levels DMP metabolites measured in urine.[48]

There are some key limitations to discuss regarding the CHC analysis, the foremost being the small sample which weakened the overall statistical power. Additionally, the timing of the OP exposure and asthma outcome measures for this cross-sectional analysis is something to consider. DAP metabolite levels averaged across all provided urine samples act as a proxy for a child's average OP exposure throughout the year 2011; however, the asthma diagnosis have occurred at a point prior to 2011. Since it is not possible to assess exposures that precede the outcome for this cohort, this analysis assumes that children on the higher end of OP exposure in 2011 will generally have been on the higher end of OP exposure over their life course to address this timing limitation.

4.5 Conclusion

This analysis demonstrates how measuring a strategically chosen cytokine profile can provide insight into many facets of respiratory-associated immune and inflammatory signaling. Using a systems-based approach to build and interpret cytokine profiles, allows researchers to establish a balance between cost and context through minimizing the number of analytes while maximizing coverage of signaling pathways associated with the system of interest.

While this data had inherent limitations due to the small sample size and large proportion of analytes below the limit of detection, the use of Bayesian mixed effects models fit using MC-MC Hamiltonian sampling methods allowed valuable insight to be gleaned from the available data. Specifically, modeling the biomarker concentrations as a normal distribution based off of other driving factors (i.e. season of sample collection, age, sex, etc.) and prior knowledge of a range in which the values fall provided a more complete picture of the data compared to treating all data below the limit of detection as a single value (e.g. LOD/√2). Moreover, the use of LOD/√2 imputation methods tends to bias the mean upwards and significantly underestimate uncertainty, but these two limitations are effectively addressed by using Bayesian MC-MC approaches. The modeled distribution also allows for more complexity in subsequent analyses compared with other methods such as dividing the exposures into quantiles. Considering these advantages, this

approach to handling data with substantial measurements below the limit of detection is promising, especially if scaled up to larger sample sizes which could ease interpretation of the results through helping to refine the modeled distributions.

The longitudinal follow-up of this cohort and use of linear mixed effects regressions expanded our understanding of both child variability and seasonal trends. Capturing this between and within subject variability is a powerful rationalization for using these mixed effects approaches as it provides insight into exposure and response differences across time and across populations that may be valuable for developing effective prevention strategies aimed at alleviating environmentally exacerbated health impacts. The results indicated that harnessing seasonal granularity is vital to fully understanding environmental determinants of inflammation for children in the lower Yakima Valley. This highlights the need for repeated measures of inflammatory markers when conducting future studies assessing early-life pesticide exposure in agricultural settings.

FUTURE DIRECTIONS

The results obtained using this approach promise to contribute to the future development of cytokine fingerprints that act as biomarkers of exposure and response. Drawing a connection between exposures and biomarkers provides mechanistic insight has implications for improving prevention, diagnostic, and treatment methods. Furthermore, this cohort's longitudinal follow-up provided a unique opportunity to analyze how health outcomes related to environmental stressors vary seasonally, an important factor to consider due to seasonal fluctuations in exposures unique to agricultural communities.

Looking forward, the methodology used in this research can be applied to the field of cumulative impacts. In addition to the data reported in this thesis, markers of other chemical (i.e. phthalates, metals) and non-chemical (i.e. psychosocial stress) stressors have been measured for this cohort. Historically, modeling the dose-response relationship of a single agent and outcome was the primary method for investigating diseases with suspected environmental etiologies; however, this approach does not accurately reflect the cumulative and interactive reality of exposures faced throughout one's life course. To address this methodological gap, Christopher Wild defined the "exposome" in 2005.[70] The exposome framework is described as a "dynamic entity"

that characterizes chemical and non-chemical exposures from conception to death through integration of extensive qualitative and quantitative data. The longitudinal nature of the framework provides an effective perspective through which to explore how a community's exposures fluctuate with seasonal farming practices and evolve alongside a dynamic regulatory landscape. Therefore, it is an ideal lens through which to model the complex exposure mixture faced by children within agricultural communities. This can be done through leveraging a systems-based approach and strategically measured biomarkers to combine chemical and non-chemical exposures into a single statistical model on the basis that they act through congruent mechanisms and manifest in similar health endpoints. Evaluating the contributions of a mixture of exposures to asthma will provide valuable insight to develop appropriate public health interventions and education initiatives.

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SUPPLEMENTAL MATERIALS

1. SUPPLEMENTAL EQUATIONS

Equation 1: Bayesian MC-MC Model of Total Dialkylphosphate Urinary Metabolites (∑DAP)

$$\sum DAP \left(\frac{nmol}{mL}\right) = DMP + DMTP + DMDTP + DEP + DETP + DEDTP$$

modeled as a normal distribution, $\ln(\sum DAP) \sim N(f(x), \sigma)$

where

$$f(x) = \beta_1 \cdot season + \beta_2 \cdot (sex \cdot age) + \mu_{child}$$

β_1 = coefficient of the fixed effect due to the season of sample collection

β_2 = coefficient of the interaction between sex and age

μ_{child} = random intercept for each child, $\mu_{child} \sim N(0, e)$

σ = standard deviation of the residuals.

Equation 2: Bayesian MC-MC Model of Total Dimethyl Phosphate Urinary Metabolites (∑DMP)

$$\sum DMP\ (nmol/mL) = DMP + DMTP + DMDTP$$

modeled as a normal distribution, $\ln(\sum DMP) \sim N(f(x), \sigma)$

where

$$f(x) = \beta_1 \cdot season + \beta_2 \cdot (sex \cdot age) + \mu_{child}$$

β_1 = coefficient of the fixed effect due to the season of sample collection

β_2 = coefficient of the interaction between sex and age

μ_{child} = random intercept for each child, $\mu_{child} \sim N(0, e)$

σ = standard deviation of the residuals.

Equation 3: Bayesian MC-MC Model of Total Diethyl Phosphate Urinary Metabolites (∑DEP)

$$\sum DEP\ (nmol/mL) = DEP + DETP + DEDTP$$

modeled as a normal distribution, $\ln(\sum DEP) \sim N(f(x), \sigma)$

where

$$f(x) = \beta_1 \cdot season + \beta_2 \cdot (sex \cdot age) + \mu_{child}$$

β_1 = coefficient of the fixed effect due to the season of sample collection

β_2 = coefficient of the interaction between sex and age

μ_{child} = random intercept for each child, $\mu_{child} \sim N(0, e)$

σ = standard deviation of the residuals

Equation 4: Bayesian MC-MC Model of Plasma Cytokine Levels

$$\ln(Cytokine\ (pg/mL)) \sim N(f(x), \sigma)$$

where

$$f(x) = \beta_1 \cdot season + \beta_2 \cdot (sex \cdot age) + \mu_{child}$$

β_1 = coefficient of the fixed effect due to the season of sample collection

β_2 = coefficient of the interaction between sex and age

μ_{child} = random intercept for each child, $\mu_{child} \sim N(0, e)$

σ = standard deviation of the residuals

Equation 5: Base Binomial Logistic Regression of Asthma Status on ∑DAP

$$\ln(\mu_i/(1-\mu_i)) = \beta_0 + \beta_1 \cdot \sum DAP_i + e$$

for each child, i, where

μ = probability of having an asthma diagnosis

β_0 = intercept

β_1 = regression coefficient for ∑DAP

$\sum DAP$ = total dialkylphosphate metabolite dose term averaged across all seasons, estimated using Bayesian MC-MC techniques (see Equation 1)

e = residual error

Equation 6: Binomial Logistic Regression of Asthma Status on ∑DAP Adjusting for Covariates

$$\ln(\mu_i/(1-\mu_i)) = \beta_0 + \beta_1 \cdot \sum DAP_i + \beta_2 \cdot sex_i + \beta_3 \cdot age_i + \beta_4 \cdot parental\ occupation_i + \beta_5 \cdot parental\ asthma_i + \beta_6 \cdot household\ smoker_i + e$$

for each child, i, where

μ = probability of having an asthma diagnosis

β_0 = intercept

β_1 = regression coefficient for ∑DAP

$\sum DAP$ = total dialkylphosphate metabolite dose term averaged across all seasons, estimated using Bayesian MC-MC techniques (see Equation 1)

β_2 = regression coefficient for child's sex

β_3 = regression coefficient for child's age at time of sample collection

β_4 = regression coefficient for whether or not there is a farmworker present in the household

β_5 = regression coefficient for whether or not parent responding to the questionnaire has a history of an asthma diagnosis

β_6 = regression coefficient for whether or not the questionnaire respondent reports someone smoking in the household

e = residual error

Equation 7: Base Binomial Logistic Regression of Asthma Status on ∑DMP

$$\ln(\mu_i/(1-\mu_i)) = \beta_0 + \beta_1 \cdot \sum DMP_i + e$$

for each child, i, where

μ = probability of having an asthma diagnosis

β_0 = intercept

β_1 = regression coefficient for ∑DMP

$\sum DMP$ = total dimethyl phosphate metabolite dose term averaged across all seasons, estimated using Bayesian MC-MC techniques (see Equation 2)

e = residual error

Equation 8: Binomial Logistic Regression of Asthma Status on ∑DMP Adjusting for Covariates

$$\ln(\mu_i/(1-\mu_i)) = \beta_0 + \beta_1 \cdot \sum DMP_i + \beta_2 \cdot sex_i + \beta_3 \cdot age_i + \beta_4 \cdot parental\ occupation_i + \beta_5 \cdot parental\ asthma_i + \beta_6 \cdot household\ smoker_i + e$$

for each child, i, where

μ = probability of having an asthma diagnosis

β_0 = intercept

β_1 = regression coefficient for ∑DMP

$\sum DMP$ = total dimethyl phosphate metabolite dose term averaged across all seasons, estimated using Bayesian MC-MC techniques (see Equation 2)

β_2 = regression coefficient for child's sex

β_3 = regression coefficient for child's age at time of sample collection

β_4 = regression coefficient for whether or not there is a farmworker present in the household

β_5 = regression coefficient for whether or not parent responding to the questionnaire has a history of an asthma diagnosis

β_6 = regression coefficient for whether or not the questionnaire respondent reports someone smoking in the household

e = residual error

Equation 9: Base Binomial Logistic Regression of Asthma Status on ∑DEP

$$\ln(\mu_i/(1-\mu_i)) = \beta_0 + \beta_1 \cdot \sum DEP_i + e$$

for each child, i, where

μ = probability of having an asthma diagnosis

β_0 = intercept

β_1 = regression coefficient for ∑DEP

$\sum DEP$ = total diethyl phosphate metabolite dose term averaged across all seasons, estimated using Bayesian MC-MC techniques (see Equation 3)

e = residual error

Equation 10: Binomial Logistic Regression of Asthma Status on ∑DEP Adjusting for Covariates

$$\ln(\mu_i/(1-\mu_i)) = \beta_0 + \beta_1 \cdot \sum DEP_i + \beta_2 \cdot sex_i + \beta_3 \cdot age_i + \beta_4 \cdot parental\ occupation_i + \beta_5 \cdot parental\ asthma_i + \beta_6 \cdot household\ smoker_i + e$$

for each child, i, where

μ = probability of having an asthma diagnosis

β_0 = intercept

β_1 = regression coefficient for ∑DEP

$\sum DEP$ = total diethyl phosphate metabolite dose term averaged across all seasons, estimated using Bayesian MC-MC techniques (see Equation 3)

β_2 = regression coefficient for child's sex

β_3 = regression coefficient for child's age at time of sample collection

β_4 = regression coefficient for whether or not there is a farmworker present in the household

β_5 = regression coefficient for whether or not parent responding to the questionnaire has a history of an asthma diagnosis

β_6 = regression coefficient for whether or not the questionnaire respondent reports someone smoking in the household

e = residual error

Equation 11: Binomial Logistic Regression of Asthma Status on ∑DAP to Assess Effect Modification

$$\ln(\mu_i/(1-\mu_i)) = \beta_0 + \beta_1 \cdot \sum DAP_i + \beta_2 \cdot sex_i + \beta_3 \cdot age_i + \beta_4 \cdot parental\ occupation_i + \beta_5 \cdot parental\ asthma_i + \beta_6 \cdot household\ smoker_i + \beta_7 \cdot (\sum DAP_i \cdot sex_i) + \beta_8 \cdot (\sum DAP_i \cdot age_i) + \beta_9 \cdot (\sum DAP_i \cdot parental\ occupation_i) + \beta_{10} \cdot (\sum DAP_i \cdot parental\ asthma_i) + e$$

for each child, i, where

μ = probability of having an asthma diagnosis

β_0 = intercept

β_1 = regression coefficient for ∑DAP

$\sum DAP$ = total dialkylphosphate metabolite dose term averaged across all seasons, estimated using Bayesian MC-MC techniques (see Equation 1)

β_2 = regression coefficient for child's sex

β_3 = regression coefficient for child's age at time of sample collection

β_4 = regression coefficient for whether or not there is a farmworker present in the household

β_5 = regression coefficient for whether or not parent responding to the questionnaire has a history of an asthma diagnosis

β_6 = regression coefficient for whether or not the questionnaire respondent reports someone smoking in the household

β_7 = coefficient of the interaction between ∑DAP and the child's sex

β_8 = coefficient of the interaction between ∑DAP and the child's age

β_9 = coefficient of the interaction between ∑DAP and the presence of a farmworker in the household

β_{10} = coefficient of the interaction between ∑DAP and parental history of asthma

e = residual error

Equation 12: Binomial Logistic Regression of Asthma Status on ∑DMP to Assess Effect Modification

$$\ln(\mu_i/(1-\mu_i)) = \beta_0 + \beta_1 \cdot \sum DMP_i + \beta_2 \cdot sex_i + \beta_3 \cdot age_i + \beta_4 \cdot parental\ occupation_i + \beta_5 \cdot parental\ asthma_i + \beta_6 \cdot household\ smoker_i + \beta_7 \cdot (\sum DMP_i \cdot sex_i) + \beta_8 \cdot (\sum DMP_i \cdot age_i) + \beta_9 \cdot (\sum DMP_i \cdot parental\ occupation_i) + \beta_{10} \cdot (\sum DMP_i \cdot parental\ asthma_i) + e$$

for each child, i, where

μ = probability of having an asthma diagnosis

β_0 = intercept

β_1 = regression coefficient for ∑DMP

$\sum DMP$ = total dimethyl phosphate metabolite dose term averaged across all seasons, estimated using Bayesian MC-MC techniques (see Equation 2)

β_2 = regression coefficient for child's sex

β_3 = regression coefficient for child's age at time of sample collection

β_4 = regression coefficient for whether or not there is a farmworker present in the household

β_5 = regression coefficient for whether or not parent responding to the questionnaire has a history of an asthma diagnosis

β_6 = regression coefficient for whether or not the questionnaire respondent reports someone smoking in the household

β_7 = coefficient of the interaction between ∑DMP and the child's sex

β_8 = coefficient of the interaction between ∑DMP and the child's age

β_9 = coefficient of the interaction between ∑DMP and the presence of a farmworker in the household

β_{10} = coefficient of the interaction between ∑DMP and parental history of asthma

e = residual error

Equation 13: Binomial Logistic Regression of Asthma Status on ∑DEP to Assess Effect Modification

$$\ln(\mu_i/(1-\mu_i)) = \beta_0 + \beta_1 \cdot \sum DEP_i + \beta_2 \cdot sex_i + \beta_3 \cdot age_i + \beta_4 \cdot parental\ occupation_i + \beta_5 \cdot parental\ asthma_i + \beta_6 \cdot household\ smoker_i + \beta_7 \cdot (\sum DEP_i \cdot sex_i) + \beta_8 \cdot (\sum DEP_i \cdot age_i) + \beta_9 \cdot (\sum DEP_i \cdot parental\ occupation_i) + \beta_{10} \cdot (\sum DEP_i \cdot parental\ asthma_i) + e$$

for each child, i, where

μ = probability of having an asthma diagnosis

β_0 = intercept

β_1 = regression coefficient for ∑DEP

$\sum DEP$ = total diethyl phosphate metabolite dose term averaged across all seasons, estimated using Bayesian MC-MC techniques (see Equation 3)

β_2 = regression coefficient for child's sex

β_3 = regression coefficient for child's age at time of sample collection

β_4 = regression coefficient for whether or not there is a farmworker present in the household

β_5 = regression coefficient for whether or not parent responding to the questionnaire has a history of an asthma diagnosis

β_6 = regression coefficient for whether or not the questionnaire respondent reports someone smoking in the household

β_7 = coefficient of the interaction between ∑DEP and the child's sex

β_8 = coefficient of the interaction between ∑DEP and the child's age

β_9 = coefficient of the interaction between ∑DEP and the presence of a farmworker in the household

β_{10} = coefficient of the interaction between ∑DEP and parental history of asthma

e = residual error

Equation 14: Base Linear Mixed Effects Model of Cytokine Level on ∑DAP

$Cytokine\ (pg/mL) \sim\ = \beta_0 + \beta_1 \cdot \sum DAP + \mu_{child}$

β_0 = intercept

β_1 = coefficient of the fixed effect due to urinary ∑DAP level

$\sum DAP$ = total dialkylphosphate metabolite dose term, estimated for each season for each child using Bayesian MC-MC techniques (see Equation 1)

μ_{child} = random intercept for each child, $\mu_{child} \sim N(0, e)$

σ = standard deviation of the residuals

Equation 15: Adjusted Linear Mixed Effects Model of Cytokine Level on ∑DAP

$$Cytokine\ (pg/mL) = \beta_0 + \beta_1 \cdot \sum DAP + \beta_2 \cdot sex + \beta_3 \cdot age + \beta_4 \cdot parental\ occupation + \mu_{child}$$

β_0 = intercept

β_1 = coefficient of the fixed effect due to urinary ∑DAP level

$\sum DAP$ = total dialkylphosphate metabolite dose term, estimated for each season for each child using Bayesian MC-MC techniques (see Equation 1)

β_1 = coefficient of the fixed effect due to the child's sex

β_3 = coefficient of the fixed effect due to the child's age

β_4 = coefficient of the fixed effect due to the presence of a farmworker in the household

μ_{child} = random intercept for each child, $\mu_{child} \sim N(0, e)$

σ = standard deviation of the residuals

Equation 16: Base Linear Mixed Effects Model of Cytokine Level on ∑DMP

$$Cytokine\ (pg/mL) = \beta_0 + \beta_1 \cdot \sum DMP + \mu_{child}$$

β_0 = intercept

β_1 = coefficient of the fixed effect due to urinary ∑DMP level

$\sum DMP$ = total dimethyl phosphate metabolite dose term, estimated for each season for each child using Bayesian MC-MC techniques (see Equation 2)

μ_{child} = random intercept for each child, $\mu_{child} \sim N(0, e)$

σ = standard deviation of the residuals

Equation 17: Adjusted Linear Mixed Effects Model of Cytokine Level on ∑DMP

$$Cytokine\ (pg/mL) = \beta_0 + \beta_1 \cdot \sum DMP + \beta_2 \cdot sex + \beta_3 \cdot age + \beta_4 \cdot parental\ occupation + \mu_{child}$$

β_0 = intercept

β_1 = coefficient of the fixed effect due to urinary ∑DMP level

$\sum DMP$ = total dimethyl phosphate metabolite dose term, estimated for each season for each child using Bayesian MC-MC techniques (see Equation 2)

β_2 = coefficient of the fixed effect due to the child's sex

β_3 = coefficient of the fixed effect due to the child's age

β_4 = coefficient of the fixed effect due to the presence of a farmworker in the household

μ_{child} = random intercept for each child, $\mu_{child} \sim N(0, e)$

σ = standard deviation of the residuals

Equation 18: Base Linear Mixed Effects Model of Cytokine Level on ∑DEP

$$Cytokine\ (pg/mL) = \beta_0 + \beta_1 \cdot \sum DEP + \mu_{child}$$

β_0 = intercept

β_1 = coefficient of the fixed effect due to urinary ∑DEP level

$\sum DEP$ = total diethyl phosphate metabolite dose term, estimated for each season for each child using Bayesian MC-MC techniques (see Equation 3)

μ_{child} = random intercept for each child, $\mu_{child} \sim N(0, e)$

σ = standard deviation of the residuals

Equation 19: Adjusted Linear Mixed Effects Model of Cytokine Level on ∑DEP

$$Cytokine\ (pg/mL) = \beta_0 + \beta_1 \cdot \sum DEP + \beta_2 \cdot sex + \beta_3 \cdot age + \beta_4 \cdot parental\ occupation + \mu_{child}$$

β_0 = intercept

β_1 = coefficient of the fixed effect due to urinary ∑DEP level

$\sum DEP$ = total diethyl phosphate metabolite dose term, estimated for each season for each child using Bayesian MC-MC techniques (see Equation 3)

β_2 = coefficient of the fixed effect due to the child's sex

β_3 = coefficient of the fixed effect due to the child's age

β_4 = coefficient of the fixed effect due to the presence of a farmworker in the household

μ_{child} = random intercept for each child, $\mu_{child} \sim N(0, e)$

σ = standard deviation of the residuals

Equation 20: Linear Mixed Effects Model of Cytokine Level on ∑DAP to Assess Effect Modification

$$Cytokine\ (pg/mL) = \beta_0 + \beta_1 \cdot \sum DAP + \beta_2 \cdot sex + \beta_3 \cdot age + \beta_4 \cdot parental\ occupation + \beta_5 \cdot (\sum DAP \cdot sex) + \beta_6 \cdot (\sum DAP \cdot age) + \beta_7 \cdot (\sum DAP \cdot parental\ occupation) + \mu_{child}$$

β_0 = intercept

β_1 = coefficient of the fixed effect due urinary ∑DMP level

$\sum DAP$ = total dialkylphosphate metabolite dose term, estimated for each season for each child using Bayesian MC-MC techniques (see Equation 1)

β_2 = coefficient of the fixed effect due to the child's sex

β_3 = coefficient of the fixed effect due to the child's age

β_4 = coefficient of the fixed effect due to the presence of a farmworker in the household

β_5 = coefficient of the interaction between ∑DAP and the child's sex

β_6 = coefficient of the interaction between ∑DAP and the child's age

β_7 = coefficient of the interaction between ∑DAP and the presence of a farmworker in the household

μ_{child} = random intercept for each child, $\mu_{child} \sim N(0, e)$

σ = standard deviation of the residuals

Equation 21: Linear Mixed Effects Model of Cytokine Level on ∑DMP to Assess Effect Modification

$$Cytokine\ (pg/mL) = \beta_0 + \beta_1 \cdot \sum DMP + \beta_2 \cdot sex + \beta_3 \cdot age + \beta_4 \cdot parental\ occupation + \beta_5 \cdot (\sum DMP \cdot sex) + \beta_6 \cdot (\sum DMP \cdot age) + \beta_7 \cdot (\sum DMP \cdot parental\ occupation) + \mu_{child}$$

β_0 = intercept

β_1 = coefficient of the fixed effect due urinary ∑DMP level

$\sum DMP$ = total dimethyl phosphate metabolite dose term, estimated for each season for each child using Bayesian MC-MC techniques (see Equation 2)

β_2 = coefficient of the fixed effect due to the child's sex

β_3 = coefficient of the fixed effect due to the child's age

β_4 = coefficient of the fixed effect due to the presence of a farmworker in the household

β_5 = coefficient of the interaction between ∑DMP and the child's sex

β_6 = coefficient of the interaction between ∑DMP and the child's age

β_7 = coefficient of the interaction between ∑DMP and the presence of a farmworker in the household

μ_{child} = random intercept for each child, $\mu_{child} \sim N(0, e)$

σ = standard deviation of the residuals

Equation 22: Linear Mixed Effects Model of Cytokine Level on ∑DEP to Assess Effect Modification

$$Cytokine\ (pg/mL) = \beta_0 + \beta_1 \cdot \sum DEP + \beta_2 \cdot sex + \beta_3 \cdot age + \beta_4 \cdot parental\ occupation + \beta_5 \cdot (\sum DEP \cdot sex) + \beta_6 \cdot (\sum DEP \cdot age) + \beta_7 \cdot (\sum DEP \cdot parental\ occupation) + \mu_{child}$$

β_0 = intercept

β_1 = coefficient of the fixed effect due urinary ∑DMP level

$\sum DEP$ = total diethyl phosphate metabolite dose term, estimated for each season for each child using Bayesian MC-MC techniques (see Equation 3)

β_2 = coefficient of the fixed effect due to the child's sex

β_3 = coefficient of the fixed effect due to the child's age

β_4 = coefficient of the fixed effect due to the presence of a farmworker in the household

β_5 = coefficient of the interaction between ∑DEP and the child's sex

β_6 = coefficient of the interaction between ∑DEP and the child's age

β_7 = coefficient of the interaction between ∑DEP and the presence of a farmworker in the household

μ_{child} = random intercept for each child, $\mu_{child} \sim N(0, e)$

σ = standard deviation of the residuals

2. SUPPLEMENTAL TABLES

Supplemental Table 1. Results of likelihood ratio tests comparing base models to the models adjusted for covariates for logistic binomial regressions

Model	Outcome	Predictor	Residual Deviance		p-value
			Base Model	Adjusted Model	
Logistic Regression	Child Asthma Status	∑DAP	64.8	62.2	0.765
		∑DMP	64.9	62.1	0.732
		∑DEP	64.6	62	0.759

Supplemental Table 2. Results of likelihood ratio tests comparing base models to the models adjusted for covariates for linear mixed effects regressions

Model	Outcome	Predictor	AIC		Chi-squared	p-value
			Base Model	Adjusted Model		
Linear Mixed Effects Regression	IL-1β	∑DAP	314	286	33.9	0.00252
	IL-5		-204	-237	38.9	0.014
	IL-6		-47.3	-92.9	51.6	0.00331
	IL-8		866	764	108	0.0000449
	IL-17A		8.35	-80.1	94.5	0.00346
	IFN-γ		1350	1300	54.4	0.000997
	MCP-1		1680	1500	181	0.00355
	TNF-α		-130	-242	118	0.00283
Linear Mixed Effects Regression	IL-1β	∑DMP	308	275	38.7	0.00485
	IL-5		-208	-243	41.1	0.0156
	IL-6		-58.6	-103	50.5	0.00789
	IL-8		857	759	104	0.000599
	IL-17A		0.785	-84	90.8	0.0041
	IFN-γ		1340	1280	66.1	0.00193
	MCP-1		1660	1490	179	0.00538
	TNF-α		-147	-255	113	0.00779
Linear Mixed Effects Regression	IL-1β	∑DEP	318	293	31	0.00321
	IL-5		-190	-218	34.3	0.016
	IL-6		-36.6	-70.7	40.1	0.00443
	IL-8		869	765	109	0.0000172
	IL-17A		14.4	-69	89.4	0.003
	IFN-γ		1350	1310	51.4	0.000389
	MCP-1		1690	1530	166	0.00399
	TNF-α		-120	-216	101	0.00194

Supplemental Table 3. Results from Bayesian mixed effects models fit via MC-MC Hamiltonian sampling methods to model urinary DAP metabolite levels

	log(Total DAP) | cens(censor, log(Total DAP Upper Limit))		log(Total DMP) | cens(censor, log(Total DMP Upper Limit))		log(Total DEP) | cens(censor, log(Total DEP Upper Limit))	
Predictors	*Estimates*	*CI (95%)*	*Estimates*	*CI (95%)*	*Estimates*	*CI (95%)*
Season (Pre-thinning)	-1.96	-2.69 – -1.23	-2.46	-3.18 – -1.74	-3.09	-3.88 – -2.28
Season (Non-spray)	-2.01	-2.78 – -1.21	-2.45	-3.24 – -1.67	-3.18	-4.01 – -2.31
Season (Thinning)	-1.83	-2.58 – -1.07	-2.35	-3.11 – -1.60	-3.04	-3.86 – -2.21
Occupation (Non-farmworker)	0.08	-0.13 – 0.29	0.03	-0.18 – 0.24	0.18	-0.04 – 0.41
Sex:Age (Female:Age)	-0.05	-0.13 – 0.02	-0.06	-0.13 – 0.02	-0.06	-0.14 – 0.02
Sex:Age (Male:Age)	-0.05	-0.12 – 0.02	-0.06	-0.13 – 0.01	-0.05	-0.13 – 0.02

Supplemental Table 4. Results from Bayesian mixed effects models fit via MC-MC Hamiltonian sampling methods to model plasma cytokine levels

	log(IL-1beta) | cens(censor)		log(IL-5) | cens(censor)		log(IL-6) | cens(censor)		log(IL-8) | cens(censor)		log(IL-17A) | cens(censor)		log(IFN-gamma) | cens(censor)		log(MCP-1) | cens(censor)		log(TNF-alpha) | cens(censor)	
Predictors	*Estimates*	*CI (95%)*	*Estimates*	*CI (95%)*	*Estimates*	*CI (95%)*	*Estimates*	*CI (95%)*	*Estimates*	*CI (95%)*	*Estimates*	*CI (95%)*	*Estimates*	*CI (95%)*	*Estimates*	*CI (95%)*
Season (Non-spray)	-0.62	-2.09 – 0.83	-1.97	-3.34 – -0.70	-0.58	-1.59 – 0.43	1.43	0.56 – 2.30	-0.29	-1.26 – 0.70	2.90	2.08 – 3.73	5.29	4.66 – 5.90	-0.26	-0.96 – 0.46
Season (Pre-thinning)	-0.16	-1.55 – 1.20	-1.81	-3.10 – -0.61	-0.41	-1.37 – 0.54	1.91	1.09 – 2.73	-0.16	-1.08 – 0.78	3.35	2.56 – 4.12	5.35	4.75 – 5.92	-0.12	-0.78 – 0.56
Season (Thinning)	-0.65	-2.08 – 0.73	-1.68	-2.99 – -0.46	-0.64	-1.61 – 0.33	1.76	0.92 – 2.60	-0.18	-1.12 – 0.78	2.93	2.13 – 3.72	5.36	4.75 – 5.94	-0.25	-0.92 – 0.45
Sex:Age (Female:Age)	-0.04	-0.18 – 0.10	0.06	-0.06 – 0.19	0.00	-0.10 – 0.10	-0.04	-0.12 – 0.05	0.03	-0.06 – 0.12	-0.06	-0.14 – 0.02	-0.05	-0.11 – 0.01	0.01	-0.06 – 0.07
Sex:Age (Male:Age)	-0.03	-0.16 – 0.10	0.07	-0.04 – 0.19	0.00	-0.09 – 0.09	-0.01	-0.09 – 0.07	0.03	-0.05 – 0.12	-0.04	-0.11 – 0.03	-0.03	-0.09 – 0.02	0.02	-0.04 – 0.08

Supplemental Table 5. Summary of effect modification assessment through assessment of interaction terms and their p-values.

Model	Outcome	Predictor	Covariate	p-value	Evidence of Effect Modification? (p<0.1)
Logistic Regression	Child Asthma Status	∑DAP	Sex	0.495	No
			Age	0.472	No
			Presence of farmworker in the household	0.458	No
			Parental asthma status	0.461	No
Logistic Regression	Child Asthma Status	∑DMP	Sex	0.421	No
			Age	0.384	No
			Presence of farmworker in the household	0.498	No
			Parental asthma status	0.5	No

Logistic Regression	Child Asthma Status	∑DEP	Sex	0.49	No
			Age	0.178	No
			Presence of farmworker in the household	0.391	No
			Parental asthma status	0.501	No
Linear Mixed Effects Regression	IL-1β	∑DAP	Season	0.558	No
			Sex	0.638	No
			Age	0.673	No
			Presence of farmworker in the household	0.665	No
Linear Mixed Effects Regression	IL-1β	∑DMP	Season	0.57	No
			Sex	0.613	No
			Age	0.634	No
			Presence of farmworker in the household	0.65	No
Linear Mixed Effects Regression	IL-1β	∑DEP	Season	0.48	No
			Sex	0.482	No
			Age	0.651	No
			Presence of farmworker in the household	0.648	No
Linear Mixed Effects Regression	IL-5	∑DAP	Season	0.614	No
			Sex	0.479	No
			Age	0.558	No
			Presence of farmworker in the household	0.161	No
Linear Mixed Effects Regression	IL-5	∑DMP	Season	0.496	No
			Sex	0.48	No
			Age	0.52	No
			Presence of farmworker in the household	0.271	No
Linear Mixed Effects Regression	IL-5	∑DEP	Season	0.563	No
			Sex	0.516	No
			Age	0.464	No
			Presence of farmworker in the household	0.192	No
Linear Mixed Effects Regression	IL-6	∑DAP	Season	0.656	No
			Sex	0.677	No
			Age	0.729	No
			Presence of farmworker in the household	0.485	No
Linear Mixed	IL-6	∑DMP	Season	0.632	No
			Sex	0.606	No

Effects Regression			Age	0.688	No
			Presence of farmworker in the household	0.435	No
Linear Mixed Effects Regression	IL-6	∑DEP	Season	0.663	No
			Sex	0.673	No
			Age	0.593	No
			Presence of farmworker in the household	0.551	No
Linear Mixed Effects Regression	IL-8	∑DAP	Season	0.265	No
			Sex	0.567	No
			Age	0.452	No
			Presence of farmworker in the household	0.574	No
Linear Mixed Effects Regression	IL-8	∑DMP	Season	0.28	No
			Sex	0.526	No
			Age	0.518	No
			Presence of farmworker in the household	0.524	No
Linear Mixed Effects Regression	IL-8	∑DEP	Season	0.264	No
			Sex	0.494	No
			Age	0.502	No
			Presence of farmworker in the household	0.539	No
Linear Mixed Effects Regression	IL-17A	∑DAP	Season	0.642	No
			Sex	0.498	No
			Age	0.615	No
			Presence of farmworker in the household	0.391	No
Linear Mixed Effects Regression	IL-17A	∑DMP	Season	0.676	No
			Sex	0.602	No
			Age	0.632	No
			Presence of farmworker in the household	0.359	No
Linear Mixed Effects Regression	IL-17A	∑DEP	Season	0.661	No
			Sex	0.41	No
			Age	0.505	No
			Presence of farmworker in the household	0.377	No
Linear Mixed Effects Regression	IFN-γ	∑DAP	Season	0.334	No
			Sex	0.504	No
			Age	0.54	No
			Presence of farmworker	0.519	No

			in the household		
Linear Mixed Effects Regression	IFN-γ	∑DMP	Season Sex Age Presence of farmworker in the household	0.352 0.56 0.53 0.588	No No No No
Linear Mixed Effects Regression	IFN-γ	∑DEP	Season Sex Age Presence of farmworker in the household	0.174 0.468 0.243 0.254	No No No No
Linear Mixed Effects Regression	MCP-1	∑DAP	Season Sex Age Presence of farmworker in the household	0.363 0.477 0.253 0.695	No No No No
Linear Mixed Effects Regression	MCP-1	∑DMP	Season Sex Age Presence of farmworker in the household	0.376 0.463 0.36 0.68	No No No No
Linear Mixed Effects Regression	MCP-1	∑DEP	Season Sex Age Presence of farmworker in the household	0.258 0.472 0.159 0.669	No No No No
Linear Mixed Effects Regression	TNF-α	∑DMP	Season Sex Age Presence of farmworker in the household	0.449 0.664 0.61 0.779	No No No No
Linear Mixed Effects Regression	TNF-α	∑DMP	Season Sex Age Presence of farmworker in the household	0.513 0.635 0.584 0.763	No No No No
Linear Mixed Effects Regression	TNF-α	∑DEP	Season Sex Age Presence of farmworker in the household	0.485 0.645 0.57 0.675	No No No No

Supplemental Table 4. Comparison of CHC urinary ∑DMP and ∑DEP metabolite concentrations to NHANES urinary ∑DMP and ∑DEP metabolite concentrations. Geometric means calculated with all values below the LOD imputed as LOD divided by the square root of 2. Values are not adjusted for creatinine.

				Geometric Mean & 95% CI (nmol/mL)	
	Study Year	Location	Age	∑DMP	∑DEP
CHC	2011	Lower Yakima Valley, WA	7-13 years	0.074 (0.068, 0.080)	0.037 (0.030, 0.045)
NHANES	2011-2012	United States	6-11years	0.038 (0.036, 0.041)	0.020 (0.020, 0.025)

3. R VERSION 4.3.3 CODE

```
---
title: "Thesis"
output: html_document
date: "2025-06-08"
---

```{r setup, include=FALSE, message=FALSE}
#Code adapted from original script provided by Bill Griffith
knitr::opts_chunk$set(echo = FALSE, message = FALSE, warning = FALSE, comment = "")
library(brms)
library(tidybayes)
library(mediation)
library(lmerTest)
library(dplyr)
library(ggplot2)
library(patchwork)
library(sjPlot)
library(gtsummary)
library(jtools)
```

```{r Functions for Modeling Cytokines Below LOD}

#Function to get data for a specific cytokine and set-up up censoring for Bayesian MCMC mixed effects model
datasetup_cyt  <- function ( group, v.cen, v.new  ){
  # Set up Data for brm by summing variables in group
  # data frames for  variables in group
  z.cen  <- v.cen[ , match( group, names(v.cen) ), drop=F ] # Censoring indicator
  z.new  <- v.new[ , match( group, names(v.new) ), drop=F ] # now has only results of analyses
  y.cen  <- t(apply( z.cen, 1, function(x){            #  indicates any values below,within,above LODs
              return( c( sum(x=='l')!=0, sum(x=='n')!=0, sum(x=='u')!=0 ) )
            }))
  typ    <- apply( y.cen, 1, function(x){      # 7 patterns for types of censoring, within range values
            if(  x[1] & !x[2] & !x[3] ) typ<-1
            if( !x[1] &  x[2] & !x[3] ) typ<-2
            if( !x[1] & !x[2] &  x[3] ) typ<-3
            if(  x[1] &  x[2] & !x[3] ) typ<-4
            if(  x[1] & !x[2] &  x[3] ) typ<-5
            if( !x[1] &  x[2] &  x[3] ) typ<-6
            if(  x[1] &  x[2] &  x[3] ) typ<-7
            return(typ)  })
  # use typ to set up data for brm
  w  <- NULL
```
```

```
  for( i in 1:nrow(z.cen) ){
   if( typ[i]==1 ){  dt <- sum(   z.new[i,z.cen[i,]=='l'] )
                censor <- -1
                dt2  <- dt            }
   if( typ[i]==2 ){  dt <- sum(   z.new[i,z.cen[i,]=='n'] )
                censor <- 0
                dt2  <- dt            }
   if( typ[i]==3 ){  dt <- sum(   z.new[i,z.cen[i,]=='u'] )
                censor <- 1
                dt2  <- dt            }
   if( typ[i]==4 ){  dt <- sum(   z.new[i,z.cen[i,]=='n'] )
                censor <- 2
                dt2  <- dt+sum(z.new[i,z.cen[i,]=='l'])  }
   if( typ[i]==5 ){  dt <- sum(   z.new[i,z.cen[i,]=='u'] )
                censor <- 1
                dt2  <- dt            }
   if( typ[i]==6 ){  dt <- sum(   z.new[i,z.cen[i,]=='n'|z.cen[i,]=='u'] )
                censor <- 1
                dt2  <- dt            }
   if( typ[i]==7 ){  dt <- sum(   z.new[i,z.cen[i,]=='n'|z.cen[i,]=='u'] )
                censor <- 1
                dt2  <- dt            }
   w  <- rbind( w, c(dt, censor, dt2) )
  }
  w        <- data.frame(w)
  names(w)  <- c('dt', 'censor', 'dt2')
  return( data.frame( dt=w$dt, censor=w$censor, dt2=w$dt2, v.new  ) )
}

#Function to model a specified cytokine below LOD. Preliminary analyses of cytokine
variability indicate that including season, sex, and age improve the fit of the model while
including other variables (i.e. parental occupation and household income) do not improve the
model fit.
MCMCmodelSummary_cyt  <- function ( group, v.cen, v.new, chains=4, iter=c(1000,5000)  ){
  x <- datasetup_cyt(  group, v.cen, v.new )
  # Run MCMC Model
  if( sum( x$censor==2 ) >0 ){
    bfu <- bf( log(dt) | cens( censor,log(dt2) )   ~ -1 +season +sex:age + (1|house) )
  }else{
    bfu <- bf( log(dt) | cens( censor          )   ~ -1 +season +sex:age + (1|house) )  }
  print( mcmc.time<-system.time( u   <- brm( bfu,  data=x, seed = 8,
chains=chains,iter=iter[2],warmup=iter[1] ) ) )
  print( summary(u) )
  v  <- data.frame(u)
  return(  list( time    = mcmc.time,
                 fit = u,
```

```
            mcmc_sum= summary(u),
            mcmc_rsl= data.frame(u),
            indt    = v.new) )
}


```

```{r Functions for Modeling DAP Below LOD}
#Function to get data for the desired group of urinary DAP metabolites and set-up censoring for Bayesian MCMC mixed effects models
datasetup_op  <- function ( group, v.cen, v.new  ){
  # Set up Data for brm
  # data frames phthalates in group (need to translate new names to old names since input data has old names)
  z.cen  <- v.cen[ , match( group, names(v.cen) ), drop=F ]
  z.new  <- v.new[ , match( group, names(v.new) ), drop=F ] # now has only results of analyses
  y.cen  <- t(apply( z.cen, 1, function(x){           # T-F indicates any values below LOD, within range, or ablove LOD
              return( c( sum(x=='l')!=0, sum(x=='n')!=0, sum(x=='u')!=0 ) )
            }))
  typ    <- apply( y.cen, 1, function(x){      # 7 patterns for types of censoring, within range values
            if(  x[1] & !x[2] & !x[3] ) typ<-1
            if( !x[1] &  x[2] & !x[3] ) typ<-2
            if( !x[1] & !x[2] &  x[3] ) typ<-3
            if(  x[1] &  x[2] & !x[3] ) typ<-4
            if(  x[1] & !x[2] &  x[3] ) typ<-5
            if( !x[1] &  x[2] &  x[3] ) typ<-6
            if(  x[1] &  x[2] &  x[3] ) typ<-7
            return(typ)  })
  # use typ to set up data for brm
  w  <- NULL
  for( i in 1:nrow(z.cen) ){
    if( typ[i]==1 ){  dt <- sum(   z.new[i,z.cen[i,]=='l'] )
                censor <- -1
                dt2  <- dt            }
    if( typ[i]==2 ){  dt <- sum(   z.new[i,z.cen[i,]=='n'] )
                censor <- 0
                dt2  <- dt            }
    if( typ[i]==3 ){  dt <- sum(   z.new[i,z.cen[i,]=='u'] )
                censor <- 1
                dt2  <- dt            }
    if( typ[i]==4 ){  dt <- sum(   z.new[i,z.cen[i,]=='n'] )
                censor <- 2
                dt2  <- dt+sum(z.new[i,z.cen[i,]=='l'])  }
    if( typ[i]==5 ){  dt <- sum(   z.new[i,z.cen[i,]=='u'] )
```

```
                censor <- 1
                dt2  <- dt            }
   if( typ[i]==6 ){  dt <- sum(   z.new[i,z.cen[i,]=='n'|z.cen[i,]=='u'] )
                censor <- 1
                dt2  <- dt            }
   if( typ[i]==7 ){  dt <- sum(   z.new[i,z.cen[i,]=='n'|z.cen[i,]=='u'] )
                censor <- 1
                dt2  <- dt            }
   w  <- rbind( w, c(dt, censor, dt2) )
  }
  w        <- data.frame(w)
  names(w)  <- c('dt', 'censor', 'dt2')
  return( data.frame( dt=w$dt, censor=w$censor, dt2=w$dt2,
             occ=v.new$occ, rsp=v.new$rsp, sea=v.new$sea, day=v.new$day, sex=v.new$sex,
age=v.new$age_imp,
             house=v.new$house,id=v.new$id ) )
}

#FUnction to model a specified group of metabolites below LOD. Since previous studies have
indicated higher pesticide exposure levels in children of farmworkers, parental occupation has
been included in this analysis in addition to season, sex, and age.
MCMCmodelSummary_op  <- function ( group, v.cen, v.new, och, chains=4,
iter=c(1000,5000)  ){
  x <- datasetup_op(  group, v.cen, v.new )
  # Run MCMC Model
  if( sum( x$censor==2 ) >0 ){
    bfu <- bf( log(dt) | cens( censor,log(dt2) )   ~ -1 +sea +occ +sex:age + (1|house) )
  }else{
    bfu <- bf( log(dt) | cens( censor          )   ~ -1 +sea +occ +sex:age + (1|house) )  }
  print( mcmc.time<-system.time( u   <- brm( bfu,  data=x, seed = 8,
chains=chains,iter=iter[2],warmup=iter[1] ) ) )
  print( summary(u) )
  v  <- data.frame(u)
  return(  list( mcmc.time, fit=u,
                      mcmc_sum= summary(u),
                      mcmc_rsl= data.frame(u) ) )
}

```

```{r Functions for Regressing Asthma Status on DAP Metabolite Dose}

#Function to run a base logistic regression and an adjusted logistic regression. The function also
compares the two models using a chi-squared test. The function returns the slope, standard
deviation, and p-value for the DAP dose term for both models. It also returns the deviance of the
2 models and the p-value corresponding to the chi-squared test.
```

```
glm.lgstc_op  <- function( mcmc, indt ){
   mcmc.nm     <- names(mcmc$mcmc_rsl)
   mcmc.house  <- as.double( substring(mcmc.nm[ 8:105 ],9,nchar(mcmc.nm[ 8:105 ])-11) )
   hxp         <- indt[ match(mcmc.house,indt$house), c('house','age', 'occ', 'sex','wheeze', 'asthma',
'pasthma','smoke') ]
   hxp$rsp <- ifelse(hxp$asthma==1, "yes", "no")
   hxp$rsp     <- factor (hxp$rsp)
   hxp$sex     <- factor( hxp$sex )
   hxp$occ     <- factor( hxp$occ )
   hxp$pasthma <- factor( hxp$pasthma )
   hxp$smoke   <- factor( hxp$smoke )
   rsl <- NULL
   data.list <- data.frame(mcmc.house[2:68]) #initiate a list to compile all iterations of the dose
data used to run the models into a data frame, each iteration dose estimate for each house is
added as a single column

  glm.time<-system.time(for ( i in 1:nrow(mcmc$mcmc_rsl) ){
   tmp<- unlist( mcmc$mcmc_rsl[i,] )
   tmp <- c(tmp, 0)  #add a zero to the end of the dataframe as a makeshift baseline (i.e. for
farmworker)
   dx   <- ((3*((tmp[8]^2)/2))+sum(tmp[1:3]) +3*(tmp[ 9:75 ]+
tmp[ifelse(hxp$occ[2:68]=='Non-farmworker',4,78)] +
tmp[ifelse(hxp$sex[2:68]=='Female',5,6)]*hxp$age[2:68] ))/3  #average dose estimate across
three seasons
   rsp <- hxp$rsp[2:68]
   house <- mcmc.house[2:68]
   sex <- hxp$sex[2:68]
   occ <- factor(hxp$occ[2:68], levels = c("Non-farmworker", "Farmworker"))
   age <- hxp$age[2:68]
   pasthma <- hxp$pasthma[2:68]
   smoke <- hxp$smoke[2:68]
   data.list <- data.frame(data.list,dx)
   xg  <- data.frame(rsp,dx,house,sex,occ,age,pasthma,smoke)
   xg$dx <- exp(dx)
   fit <- glm( rsp ~ dx , family=binomial, data=xg )
   fit.cov <- glm( rsp ~ dx + sex + occ + pasthma + smoke + age, family=binomial, data=xg )
   model.comp <- anova(fit, fit.cov)
   resid.dev.base <- model.comp[1,2]
   resid.dev.cov <- model.comp[2,2]
   model.comp.p <- model.comp[2,5]

   ug  <- summary( fit )
   ug.cov  <- summary( fit.cov )

   mod.fit <- glm( rsp ~ dx +sex +occ +pasthma +smoke +age +dx:sex +dx:occ +dx:pasthma
+dx:age, family=binomial, data = xg)
```

```
        mod.p.test <- car::Anova(mod.fit, type = 3)
        mod.sex.p <- mod.p.test$`Pr(>Chisq)`[7] #p-value for effect modification by season
        mod.occ.p <- mod.p.test$`Pr(>Chisq)`[8] #p-value for effect modification by occupation
        mod.pasthma.p <- mod.p.test$`Pr(>Chisq)`[9] #p-value for effect modification by sex
        mod.age.p <- mod.p.test$`Pr(>Chisq)`[10] #p-value for effect modification by age
        rco.mod <- summary(mod.fit)
        mod.int <- rco.mod$coef[1,1] #intercept
        mod.int.p <- rco.mod$coef[1,4] #intercept p value
        slope.female <- rco.mod$coef[2,1] #female slope
        slope.male <- rco.mod$coef[2,1]+rco.mod$coef[8,1] #male slope
        slope.nfw <- rco.mod$coef[2,1] #nfw slope
        slope.fw <- rco.mod$coef[2,1] + rco.mod$coef[9,1] #fw slope
        slope.pasthmano <- rco.mod$coef[2,1] #female slope
        slope.pasthmayes <- rco.mod$coef[2,1] + rco.mod$coef[10,1] #male slope


    rsl    <- rbind(  rsl,  c( ug$coef[2,4], ug$coef[2,1], ug$coef[2,2],ug.cov$coef[2,4],
ug.cov$coef[2,1], ug.cov$coef[2,2], resid.dev.base, resid.dev.cov, model.comp.p, mod.int,
mod.int.p, mod.sex.p, slope.female, slope.male, mod.occ.p, slope.nfw, slope.fw, mod.pasthma.p,
slope.pasthmano, slope.pasthmayes, mod.age.p ) )
  })

  return( list( time= glm.time , glm=rsl, opdose.data = data.list ) )
}

#Functions to return the average of the results all of the regression iterations as well as a
confidence interval. This also summarizes the model comparisons.
glm_rsl_op<- function( glm_rsl ) {
  A.base <- sum(1*(glm_rsl[,2]>0))
  B.base <- min(A.base, length(glm_rsl[,2])-A.base)
  A.cov <- sum(1*(glm_rsl[,5]>0))
  B.cov <- min(A.cov, length(glm_rsl[,5])-A.cov)
  return( signif(
        c( p_value=2*B.base/length(glm_rsl[,2]), slope=mean(glm_rsl[,2]), sd=sd(glm_rsl[,2]),
          lo_95=mean(glm_rsl[,2])-1.96*sd(glm_rsl[,2]),
          up_95=mean(glm_rsl[,2])+1.96*sd(glm_rsl[,2]),

          p_value.cov=2*B.cov/length(glm_rsl[,5]), slope.cov=mean(glm_rsl[,5]),
sd.cov=sd(glm_rsl[,5]),
          lo_95.cov=mean(glm_rsl[,5])-1.96*sd(glm_rsl[,5]),
          up_95.cov=mean(glm_rsl[,5])+1.96*sd(glm_rsl[,5]),

          resid.deviance=mean(glm_rsl[,7]),
          resid.deviance.cov=mean(glm_rsl[,8]),
          p_value.model.comp=mean(glm_rsl[,9]),
```

```
        p_value.effmod.intercept=mean(glm_rsl[,11]), effmod.intercept=mean(glm_rsl[,10]),
effmod.intercept.sd=sd(glm_rsl[,10]),
        effmod.intercept.lo_95=mean(glm_rsl[,10])-1.96*sd(glm_rsl[,10]),
        effmod.intercept.up_95=mean(glm_rsl[,10])+1.96*sd(glm_rsl[,10]),
        p_value.sexdiff.effmod=mean(glm_rsl[,12]),
        p_value.occdiff.effmod=mean(glm_rsl[,15]),
        p_value.pasthmadiff.effmod=mean(glm_rsl[,18]),
        p_value.agediff.effmod=mean(glm_rsl[,21]),
        slope.female=mean(glm_rsl[,13]), sd.female=sd(glm_rsl[,13]),
        lo_95.female=mean(glm_rsl[,13])-1.96*sd(glm_rsl[,13]),
        up_95.female=mean(glm_rsl[,13])+1.96*sd(glm_rsl[,13]),
        slope.male=mean(glm_rsl[,14]), sd.male=sd(glm_rsl[,14]),
        lo_95.male=mean(glm_rsl[,14])-1.96*sd(glm_rsl[,14]),
        up_95.male=mean(glm_rsl[,14])+1.96*sd(glm_rsl[,14]),
        slope.nfw=mean(glm_rsl[,16]), sd.nfw=sd(glm_rsl[,16]),
        lo_95.nfw=mean(glm_rsl[,16])-1.96*sd(glm_rsl[,16]),
        up_95.nfw=mean(glm_rsl[,16])+1.96*sd(glm_rsl[,16]),
        slope.fw=mean(glm_rsl[,17]), sd.fw=sd(glm_rsl[,17]),
        lo_95.fw=mean(glm_rsl[,17])-1.96*sd(glm_rsl[,17]),
        up_95.fw=mean(glm_rsl[,17])+1.96*sd(glm_rsl[,17]),
        slope.pasthmano=mean(glm_rsl[,19]), sd.pasthmano=sd(glm_rsl[,19]),
        lo_95.pasthmano=mean(glm_rsl[,19])-1.96*sd(glm_rsl[,19]),
        up_95.pasthmano=mean(glm_rsl[,19])+1.96*sd(glm_rsl[,19]),
        slope.pasthmayes=mean(glm_rsl[,20]), sd.pasthmayes=sd(glm_rsl[,20]),
        lo_95.pasthmayes=mean(glm_rsl[,20])-1.96*sd(glm_rsl[,20]),
        up_95.pasthmayes=mean(glm_rsl[,20])+1.96*sd(glm_rsl[,20])

      ),3) )
}

```

```{r Functions for Regressing Cytokine Levels on DAP Metabolite Dose - NO SEASONAL COV}
#Function for linear mixed effect model of cytokines on OPs keeping seasonal observations discrete
lmer.cyt_op  <- function( cyt_mcmc, op_mcmc, indt ){
  cyt.nm    <- names(cyt_mcmc$mcmc_rsl)
  cyt.house  <- as.double( substring(cyt.nm[ 8:74 ],9,nchar(cyt.nm[ 8:74 ])-11) )
  op.nm     <- names(op_mcmc$mcmc_rsl)
  op.house   <- as.double( substring(op.nm[ 8:105 ],9,nchar(op.nm[ 8:105 ])-11) )
  cyt.op.mch <- match( cyt.house, op.house )
  hxp        <- indt[,c('house','age','sex','wheeze', 'asthma', 'season', 'occ')]
  hxp <- unique(hxp)
  hxp <- hxp[order(hxp$season),]
```

```
  hxp <- rbind(hxp[-(1:67), ], hxp[1:67, ])
  hxp$sex     <- factor( hxp$sex )
  hxp$season <- factor(hxp$season)
  hxp$occ <- factor(hxp$occ, levels = c("Non-farmworker", "Farmworker"))
 rsl  <-NULL
 data.list <- list() #initiate a list to compile all iterations of the data frames used to run the
models into a list of data frames

 glm.time<-system.time(
    for ( i in 1:nrow(cyt_mcmc$mcmc_rsl) ){
      tmp.cyt<- unlist( cyt_mcmc$mcmc_rsl[i,] )
      cyt.pt<- ((tmp.cyt[7]^2)/2)+tmp.cyt[2] + (tmp.cyt[ 8:74    ]+
tmp.cyt[ifelse(hxp$sex[1:67]=='Female',4,5)]*hxp$age[1:67] )  #pre-thinning term
      cyt.t<- ((tmp.cyt[7]^2)/2)+tmp.cyt[3] + (tmp.cyt[ 8:74    ]+
tmp.cyt[ifelse(hxp$sex[68:134]=='Female',4,5)]*hxp$age[68:134] )  #thinning term
      cyt.ns<- ((tmp.cyt[7]^2)/2)+tmp.cyt[1] + (tmp.cyt[ 8:74    ]+
tmp.cyt[ifelse(hxp$sex[135:201]=='Female',4,5)]*hxp$age[135:201] )  #non-spray term
      cyt.rsp<- c(cyt.pt, cyt.t, cyt.ns)  #combine three seasons
      tmp.op <- unlist( op_mcmc$mcmc_rsl[i,] )
      tmp.op <- c(tmp.op, 0) #add a zero to the end of the dataframe as a makeshift baseline (i.e.
for farmworker)
      op.pt  <- ((tmp.op[8]^2)/2)+tmp.op [1] + (tmp.op[7+cyt.op.mch]+
tmp.op[ifelse(hxp$occ[1:67]=='Non-farmworker',4,78)]+
tmp.op[ifelse(hxp$sex[1:67]=='Female',4,5)]*hxp$age[1:67] )  #pre-thinning term
      op.t  <- ((tmp.op[8]^2)/2)+tmp.op [3] + (tmp.op[7+cyt.op.mch]+
tmp.op[ifelse(hxp$occ[1:67]=='Non-farmworker',4,78)]+
tmp.op[ifelse(hxp$sex[1:67]=='Female',4,5)]*hxp$age[1:67] )  #thinning term
      op.ns  <- ((tmp.op[8]^2)/2)+tmp.op [2] + (tmp.op[7+cyt.op.mch]+
tmp.op[ifelse(hxp$occ[1:67]=='Non-farmworker',4,78)]+
tmp.op[ifelse(hxp$sex[1:67]=='Female',4,5)]*hxp$age[1:67] )  #non-spray term
      op.dx  <- c(op.pt, op.t, op.ns)  #combine three seasons
      pt_list <- rep("Pre-Thinning", 67)
      t_list <- rep("Thinning", 67)
      ns_list <- rep("Non-Spray", 67)
      sea <- c(pt_list, t_list, ns_list)
      house <- hxp$house
      sex <- hxp$sex
      occ <- hxp$occ
      age <- hxp$age
      tmp.values <- data.frame(op.dx,cyt.rsp)
      data.list[[i]] <- tmp.values #add estimated data points (still natural log transformed!) for
dap dose and cytokie levels for the current iteration to list of dataframes
      xg  <- data.frame(op.dx,cyt.rsp,house,sex,sea,occ,age)
      xg$sea <- factor(xg$sea, levels = c("Pre-Thinning", "Thinning", "Non-Spray"))
      xg$op.dx <- exp(op.dx)
      xg$cyt.rsp <- exp(cyt.rsp)
```

```
base.fit <- lmer( cyt.rsp ~ op.dx + (1|house) , data = xg, REML = FALSE) #fit base regression

cov.fit <- lmer( cyt.rsp ~ op.dx +sex +age +occ + (1|house), data = xg, REML = FALSE) #fit adjusted regression controlling for covariates
cov.p.test <- car::Anova(cov.fit, type = 3)
cov.op.chi <- cov.p.test$`Chisq`[2] #chi sq for incorporating op.dx
cov.sex.chi <- cov.p.test$`Chisq`[3] #chi sq for incorporating sex
cov.age.chi <- cov.p.test$`Chisq`[4] #chi sq for incorporating age
cov.occ.chi <- cov.p.test$`Chisq`[5] #chi sq for incorporating occupation
cov.op.p <- cov.p.test$`Pr(>Chisq)`[2] #p-value for incorporating op.dx
cov.sex.p <- cov.p.test$`Pr(>Chisq)`[3] #p-value for incorporating sex
cov.age.p <- cov.p.test$`Pr(>Chisq)`[4] #p-value for incorporating age
cov.occ.p <- cov.p.test$`Pr(>Chisq)`[5] #p-value for incorporating occupation

model.comp <- anova(base.fit,cov.fit) # compare to model only containing significnt covariates determined using Type III Wald chisquare tests
aic.base <- model.comp["base.fit", 2]
aic.cov <- model.comp["cov.fit", 2]
chisq <- model.comp[2, "Chisq"]
model.comp.p <- model.comp[2, "Pr(>Chisq)"]


rco    <- summary(base.fit)
rco.cov <- summary(cov.fit)

mod.fit <- lmer( cyt.rsp ~ op.dx +sea +sex +age +occ +op.dx:sea +op.dx:occ +op.dx:sex +op.dx:age + (1|house), data = xg)
mod.p.test <- car::Anova(mod.fit, type = 3)
mod.sea.p <- mod.p.test$`Pr(>Chisq)`[7] #p-value for effect modification by season
mod.occ.p <- mod.p.test$`Pr(>Chisq)`[8] #p-value for effect modification by occupation
mod.sex.p <- mod.p.test$`Pr(>Chisq)`[9] #p-value for effect modification by sex
mod.age.p <- mod.p.test$`Pr(>Chisq)`[10] #p-value for effect modification by age
rco.mod <- summary(mod.fit)
mod.int <- rco.mod$coef[1,1] #intercept
mod.int.p <- rco.mod$coef[1,5] #intercept p value
slope.ns <- rco.mod$coef[2,1] #ns slope
slope.pt <- rco.mod$coef[2,1]+rco.mod$coef[8,1] #pt slope
slope.t <- rco.mod$coef[2,1]+rco.mod$coef[9,1] #t slope
slope.nfw <- rco.mod$coef[2,1] #nfw slope
slope.fw <- rco.mod$coef[2,1] + rco.mod$coef[10,1] #fw slope
slope.female <- rco.mod$coef[2,1] #female slope
slope.male <- rco.mod$coef[2,1] + rco.mod$coef[11,1] #male slope
```

```
        rsl    <- rbind(  rsl, c( rco$coef[2,5], rco$coef[2,1], rco$coef[2,2], rco.cov$coef[2,5],
rco.cov$coef[2,1], rco.cov$coef[2,2], rco$coef[1,1], rco$coef[1,5], rco.cov$coef[1,1],
rco.cov$coef[1,5], aic.base, aic.cov, chisq, model.comp.p, mod.int, mod.int.p, mod.sea.p,
slope.pt, slope.t, slope.ns, mod.occ.p, slope.nfw, slope.fw, mod.sex.p, slope.female, slope.male,
mod.age.p, cov.op.p, cov.sex.p, cov.age.p, cov.occ.p, cov.op.chi, cov.sex.chi, cov.age.chi,
cov.occ.chi, rco.cov$coef[3,5], rco.cov$coef[3,1], rco.cov$coef[4,5], rco.cov$coef[4,1],
rco.cov$coef[5,5], rco.cov$coef[5,1]) )
   })

  data.avg <- Reduce(`+`, data.list) / length(data.list) #average the values in the list of data frames
to get an average value for each data point used in the iterations of regressions -- *this is the log
transformed values that are being averaged!
  return( list( time= glm.time, lm=rsl, avg.data=data.avg  ) )
}

lmer_rsl_cyt_op<- function( glm_rsl ) {
  A.base <- sum(1*(glm_rsl[,2]>0))
  B.base <- min(A.base, length(glm_rsl[,2])-A.base)
  A.cov <- sum(1*(glm_rsl[,5]>0))
  B.cov <- min(A.cov, length(glm_rsl[,5])-A.cov)
  A.male <- sum(1*(glm_rsl[,37]>0))
  B.male <- min(A.male, length(glm_rsl[,37])-A.male)
  A.age <- sum(1*(glm_rsl[,39]>0))
  B.age <- min(A.age, length(glm_rsl[,37])-A.age)
  A.fw <- sum(1*(glm_rsl[,41]>0))
  B.fw <- min(A.fw, length(glm_rsl[,37])-A.fw)
  return( signif(
        c( p_value=2*B.base/length(glm_rsl[,2]), slope=mean(glm_rsl[,2]), sd=sd(glm_rsl[,2]),
           lo_95=mean(glm_rsl[,2])-1.96*sd(glm_rsl[,2]),
           up_95=mean(glm_rsl[,2])+1.96*sd(glm_rsl[,2]),
           p_value.intercept=mean(glm_rsl[,8]), intercept=mean(glm_rsl[,7]),
sd.intercept=sd(glm_rsl[,7]),
           lo_95.intercept=mean(glm_rsl[,7])-1.96*sd(glm_rsl[,7]),
           up_95.intercept=mean(glm_rsl[,7])+1.96*sd(glm_rsl[,7]),

           p_value.cov=2*B.cov/length(glm_rsl[,5]), slope.cov=mean(glm_rsl[,5]),
sd.cov=sd(glm_rsl[,5]),
           lo_95.cov=mean(glm_rsl[,5])-1.96*sd(glm_rsl[,5]),
           up_95.cov=mean(glm_rsl[,5])+1.96*sd(glm_rsl[,5]),
           p_value.cov.intercept=mean(glm_rsl[,10]), cov.intercept=mean(glm_rsl[,9]),
sd.cov=sd(glm_rsl[,9]),
           lo_95.cov.intercept=mean(glm_rsl[,9])-1.96*sd(glm_rsl[,9]),
           up_95.cov.intercept=mean(glm_rsl[,9])+1.96*sd(glm_rsl[,9]),
           p_value.cov.male=2*B.male/length(glm_rsl[,37]), cov.male.est=mean(glm_rsl[,37]),
sd.cov.male=sd(glm_rsl[,37]),
           lo_95.cov.male=mean(glm_rsl[,37])-1.96*sd(glm_rsl[,37]),
```

```
        up_95.cov.male=mean(glm_rsl[,37])+1.96*sd(glm_rsl[,37]),
        p_value.cov.age=2*B.age/length(glm_rsl[,39]), cov.age.est=mean(glm_rsl[,39]),
sd.cov.age=sd(glm_rsl[,39]),
        lo_95.cov.age=mean(glm_rsl[,39])-1.96*sd(glm_rsl[,39]),
        up_95.cov.age=mean(glm_rsl[,39])+1.96*sd(glm_rsl[,39]),
        p_value.cov.fw=2*B.fw/length(glm_rsl[,41]), cov.fw.est=mean(glm_rsl[,41]),
sd.cov.fw=sd(glm_rsl[,41]),
        lo_95.cov.fw=mean(glm_rsl[,41])-1.96*sd(glm_rsl[,41]),
        up_95.cov.fw=mean(glm_rsl[,41])+1.96*sd(glm_rsl[,41]),

        p_value.effmod.intercept=mean(glm_rsl[,16]), effmod.intercept=mean(glm_rsl[,15]),
effmod.intercept.sd=sd(glm_rsl[,15]),
        effmod.intercept.lo_95=mean(glm_rsl[,15])-1.96*sd(glm_rsl[,15]),
        effmod.intercept.up_95=mean(glm_rsl[,15])+1.96*sd(glm_rsl[,15]),
        p_value.seasondiff.effmod=mean(glm_rsl[,17]),
        p_value.occdiff.effmod=mean(glm_rsl[,21]),
        p_value.sexdiff.effmod=mean(glm_rsl[,24]),
        p_value.agediff.effmod=mean(glm_rsl[,27]),
        slope.pt=mean(glm_rsl[,18]), sd.pt=sd(glm_rsl[,18]),
        lo_95.pt=mean(glm_rsl[,18])-1.96*sd(glm_rsl[,18]),
        up_95.pt=mean(glm_rsl[,18])+1.96*sd(glm_rsl[,18]),
        slope.t=mean(glm_rsl[,19]), sd.t=sd(glm_rsl[,19]),
        lo_95.t=mean(glm_rsl[,19])-1.96*sd(glm_rsl[,19]),
        up_95.t=mean(glm_rsl[,19])+1.96*sd(glm_rsl[,19]),
        slope.ns=mean(glm_rsl[,20]), sd.ns=sd(glm_rsl[,20]),
        lo_95.ns=mean(glm_rsl[,20])-1.96*sd(glm_rsl[,20]),
        up_95.ns=mean(glm_rsl[,20])+1.96*sd(glm_rsl[,20]),
        slope.nfw=mean(glm_rsl[,22]), sd.nfw=sd(glm_rsl[,22]),
        lo_95.nfw=mean(glm_rsl[,22])-1.96*sd(glm_rsl[,22]),
        up_95.nfw=mean(glm_rsl[,22])+1.96*sd(glm_rsl[,22]),
        slope.fw=mean(glm_rsl[,23]), sd.fw=sd(glm_rsl[,23]),
        lo_95.fw=mean(glm_rsl[,23])-1.96*sd(glm_rsl[,23]),
        up_95.fw=mean(glm_rsl[,23])+1.96*sd(glm_rsl[,23]),
        slope.female=mean(glm_rsl[,25]), sd.female=sd(glm_rsl[,25]),
        lo_95.female=mean(glm_rsl[,25])-1.96*sd(glm_rsl[,25]),
        up_95.female=mean(glm_rsl[,25])+1.96*sd(glm_rsl[,25]),
        slope.male=mean(glm_rsl[,26]), sd.male=sd(glm_rsl[,26]),
        lo_95.male=mean(glm_rsl[,26])-1.96*sd(glm_rsl[,26]),
        up_95.male=mean(glm_rsl[,26])+1.96*sd(glm_rsl[,26]),

        aic.base=mean(glm_rsl[,11]), aic.cov=mean(glm_rsl[,12]), chisq=mean(glm_rsl[,13]),
model.comp.pvalue=mean(glm_rsl[,14]),

        p_value.op.cov=mean(glm_rsl[,28]),
        p_value.sex.cov=mean(glm_rsl[,29]),
        p_value.age.cov=mean(glm_rsl[,30]),
```

```
            p_value.occ.cov=mean(glm_rsl[,31]),

            chi.op.cov=mean(glm_rsl[,32]),
            chi.sex.cov=mean(glm_rsl[,33]),
            chi.age.cov=mean(glm_rsl[,34]),
            chi.occ.cov=mean(glm_rsl[,35])

           ),3) )
}
```

```{r modeling cyt below LOD}
int.temp  <- read.csv( file='cytokine_data_cresp_20241018(in).csv' )
asthma_covariates <- read.csv( file='otherdata.csv' )
int <- merge(int.temp, asthma_covariates, by = "House", all = FALSE)
dim(int)
names(int)
summary(int[,"adjConc_negLOD"])
sum(is.na(int[,"Age"]))

cdt  <- data.frame(
  house =int$House,          sample=int$Sample, plate =int$Sample, cyt   =int$Assay,
  conc  =int$adjConc_negLOD, season=int$Season, age   =int$age_imp,    sex   =int$Sex,
  mnstrt=int$Menstruating.,  occ   =int$Occup,
  wheeze=int$CWHEEZE_EVER,   asthma=int$CASTHMA_DX,
pasthma=int$AsthmaParentalHistory, smoke=int$SmokingStatus )

cyt.nm  <- names( table(cdt$cyt) )

#il1
print("Model IL-1 Below LOD:")
x.il1       <- cdt[ cdt$cyt==cyt.nm[3], ]
x.cen.il1  <- data.frame( conc=ifelse( x.il1$conc<0, 'l', 'n' ) )
x.new.il1  <- x.il1
x.new.il1$conc <- abs( x.new.il1$conc )
dim(x.il1)
mcmc.il1  <- MCMCmodelSummary_cyt( 'conc', x.cen.il1, x.new.il1, chains=4,
iter=c(1000,6000)  )

#il4 - TOO MANY BELOW LOD -> remove from analysis
#print("Model IL-4 Below LOD:")
#x.il4       <- cdt[ cdt$cyt==cyt.nm[4], ]
```

```
#x.cen.il4  <- data.frame( conc=ifelse( x.il4$conc<0, 'l', 'n' ) )
#x.new.il4  <- x.il4
#x.new.il4$conc <- abs( x.new.il4$conc )
#dim(x.il4)
#mcmc.il4  <- MCMCmodelSummary_cyt( 'conc', x.cen.il4, x.new.il4, chains=4,
iter=c(1000,6000)  )

#il5
print("Model IL-5 Below LOD:")
x.il5       <- cdt[ cdt$cyt==cyt.nm[5], ]
x.cen.il5  <- data.frame( conc=ifelse( x.il5$conc<0, 'l', 'n' ) )
x.new.il5  <- x.il5
x.new.il5$conc <- abs( x.new.il5$conc )
dim(x.il5)
mcmc.il5  <- MCMCmodelSummary_cyt( 'conc', x.cen.il5, x.new.il5, chains=4,
iter=c(1000,6000)  )

#il6
print("Model IL-6 Below LOD:")
x.il6       <- cdt[ cdt$cyt==cyt.nm[6], ]
x.cen.il6  <- data.frame( conc=ifelse( x.il6$conc<0, 'l', 'n' ) )
x.new.il6  <- x.il6
x.new.il6$conc <- abs( x.new.il6$conc )
dim(x.il6)
mcmc.il6  <- MCMCmodelSummary_cyt( 'conc', x.cen.il6, x.new.il6, chains=4,
iter=c(1000,6000)  )

#il8
print("Model IL-8 Below LOD:")
x.il8       <- cdt[ cdt$cyt==cyt.nm[7], ]
x.cen.il8  <- data.frame( conc=ifelse( x.il8$conc<0, 'l', 'n' ) )
x.new.il8  <- x.il8
x.new.il8$conc <- abs( x.new.il8$conc )
dim(x.il8)
mcmc.il8  <- MCMCmodelSummary_cyt( 'conc', x.cen.il8, x.new.il8, chains=4,
iter=c(1000,6000)  )

#il17
print("Model IL-17A Below LOD:")
x.il17       <- cdt[ cdt$cyt==cyt.nm[2], ]
x.cen.il17  <- data.frame( conc=ifelse( x.il17$conc<0, 'l', 'n' ) )
x.new.il17  <- x.il17
x.new.il17$conc <- abs( x.new.il17$conc )
dim(x.il17)
mcmc.il17  <- MCMCmodelSummary_cyt( 'conc', x.cen.il17, x.new.il17, chains=4,
iter=c(1000,6000)  )
```

```
#ifn
print("Model IFN-gamma Below LOD:")
x.ifn       <- cdt[ cdt$cyt==cyt.nm[1], ]
x.cen.ifn  <- data.frame( conc=ifelse( x.ifn$conc<0, 'l', 'n' ) )
x.new.ifn  <- x.ifn
x.new.ifn$conc <- abs( x.new.ifn$conc )
dim(x.ifn)
mcmc.ifn  <- MCMCmodelSummary_cyt( 'conc', x.cen.ifn, x.new.ifn, chains=4,
iter=c(1000,6000)  )

#mcp
print("Model MCP-1 Below LOD:")
x.mcp       <- cdt[ cdt$cyt==cyt.nm[8], ]
x.cen.mcp  <- data.frame( conc=ifelse( x.mcp$conc<0, 'l', 'n' ) )
x.new.mcp  <- x.mcp
x.new.mcp$conc <- abs( x.new.mcp$conc )
dim(x.mcp)
mcmc.mcp  <- MCMCmodelSummary_cyt( 'conc', x.cen.mcp, x.new.mcp, chains=4,
iter=c(1000,6000)  )

#tnf
print("Model TNF-alpha Below LOD:")
x.tnf       <- cdt[ cdt$cyt==cyt.nm[9], ]
x.cen.tnf  <- data.frame( conc=ifelse( x.tnf$conc<0, 'l', 'n' ) )
x.new.tnf  <- x.tnf
x.new.tnf$conc <- abs( x.new.tnf$conc )
dim(x.tnf)
mcmc.tnf  <- MCMCmodelSummary_cyt( 'conc', x.cen.tnf, x.new.tnf, chains=4,
iter=c(1000,6000)  )

```

```{r modeling DAP metabolites below LOD}
op3  <- read.csv( 'CHC3 Urine OPM child only 20240617.csv' )
dim(op3)
names(op3)  <- c( "id", "house", "season",  "age",    "day",
                  "DEDTP",  "DMDTP",  "DETP", "DMTP",   "DEP",    "DMP" )
dap.nm      <- c( "DEDTP",  "DMDTP",  "DETP", "DMTP",   "DEP",    "DMP" )

sv3.temp  <- read.csv( 'chc3_survey_data_20240625(in).csv' )
sv3 <- merge(sv3.temp, asthma_covariates, by = "House", all = FALSE)
och  <- data.frame( house=sv3$House,
```

```
                occ  =ifelse(sv3$OCCUPATION_11==1 | sv3$OCCUPATION_21==1 |
sv3$OCCUPATION_31==1,'fw','nfw'),
                age  =sv3$ChildAge,
                sex  =ifelse( sv3$ChildGender==1,'female','male'),
                rsp  =ifelse(sv3$CASTHMA_DX==1,
                        'yes','no'),
                pasthma = sv3$AsthmaParentalHistory,
                smoke = sv3$SmokingStatus)
table( match( op3$house, och$house ) )
op3$occ  <- och$occ[  match( op3$house, och$house ) ]
op3$rsp  <- och$rsp[  match( op3$house, och$house ) ]
op3$sex  <- och$sex[  match( op3$house, och$house ) ]
op3$age  <- och$age[  match( op3$house, och$house ) ]
op3$sea  <- factor( op3$sea )
op3$day  <- factor( op3$day )
op3$sex  <- factor( op3$sex )
head(op3)

table(  u<-apply(op3,1,function(x){  return(sum(is.na(x[6:11]))) } ))
op3      <- op3[ u==0, ]        # only use data from samples where all 6 DAP metabolites were
analyzed
op3.cen  <- data.frame( ifelse( op3[ , dap.nm ] <0, 'l', 'n' ) )
op3.new  <- op3
op3.new[,dap.nm]  <- abs( op3[,dap.nm] )
house_sea_age <- unique(data.frame(house=x.new.il1$house, sea=x.new.il1$season,
age_imp=x.new.il1$age))
house_sea_age$sea <- ifelse(house_sea_age$sea == "Pre-thinning", "CP",
ifelse(house_sea_age$sea == "Non-Spray", "Non Spray", "Thinning"))
op3.new <- merge(op3.new, house_sea_age, by = c("house", "sea"), all.x = TRUE)
dim(op3)


#convert to molar concentrations nmol/mL
op3.new$DEDTP <- op3.new$DEDTP/186.23
op3.new$DETP <- op3.new$DETP/170.17
op3.new$DEP <- op3.new$DEP/154.1
op3.new$DMDTP <- op3.new$DMDTP/158.18
op3.new$DMTP <- op3.new$DMTP/142.11
op3.new$DMP <- op3.new$DMP/126.05

##DO NOT USE - ALL values are censored
print("Model Total DAP Metabolites Below LOD: ")
group    <- dap.nm
dap.mcmc <- MCMCmodelSummary_op ( group, op3.cen, op3.new, och, chains=4,
iter=c(1000,6000)  )
```

```
#48 are not censored, 246 are interval censored, and 370 are left censored
print("Model Total Dimethyl Metabolites Below LOD: ")
group  <- c('DMP','DMTP','DMDTP')
dmp.mcmc <- MCMCmodelSummary_op ( group, op3.cen, op3.new, och, chains=4,
iter=c(1000,6000)  )

#2 are not censored, 246 are interval censored, and 370 are left censored
print("Model Total Diethyl Metabolites Below LOD: ")
group  <- c('DEP','DETP','DEDTP')
dep.mcmc <- MCMCmodelSummary_op ( group, op3.cen, op3.new, och, chains=4,
iter=c(1000,6000)  )
```

```{r Regressions}
print("Research Question 1: Are averaged dialkyl OP metabolite levels associated with asthma
status for children in the lower Yakima Valley?")

#logistic regression of respiratory endpoint on dimethyl dose term
print("Summary for Total Dimethyl Metabolites: ")
dap.lgstc<- glm.lgstc_op( dap.mcmc, x.new.il1 )
glm_rsl_op( dap.lgstc$glm )

#logistic regression of respiratory endpoint on dimethyl dose term
print("Summary for Total Dimethyl Metabolites: ")
dmp.lgstc<- glm.lgstc_op( dmp.mcmc, x.new.il1 )
glm_rsl_op( dmp.lgstc$glm )

#logistic regression of respiratory endpoint on diethyl dose term
print("Summary for Total Diethyl Metabolites: ")
dep.lgstc<- glm.lgstc_op( dep.mcmc, x.new.il1 )
glm_rsl_op( dep.lgstc$glm )


print("Research Question 2: Are dialkyl OP metabolite levels associated with plasma cytokine
levels for children in the lower Yakima Valley?")

#il1

print("Summary for IL-1 and Total Metabolites: ")
il1_dap_lmer  <- lmer.cyt_op( mcmc.il1, dap.mcmc, x.new.il1 )
lm_rsl_cyt_op(il1_dap_lmer$lm)

print("Summary for IL-1 and Total Dimethyl Metabolites: ")
il1_dmp_lmer  <- lmer.cyt_op( mcmc.il1, dmp.mcmc, x.new.il1 )
lm_rsl_cyt_op(il1_dmp_lmer$lm)
```

```
print("Summary for IL-1 and Total Diethyl Metabolites: ")
il1_dep_lmer  <- lmer.cyt_op( mcmc.il1, dep.mcmc, x.new.il1 )
lm_rsl_cyt_op(il1_dep_lmer$lm)


#il5

print("Summary for IL-5 and Total Metabolites: ")
il5_dap_lmer  <- lmer.cyt_op( mcmc.il5, dap.mcmc, x.new.il5 )
lm_rsl_cyt_op(il5_dap_lmer$lm)

print("Summary for IL-5 and Total Dimethyl Metabolites: ")
il5_dmp_lmer  <- lmer.cyt_op( mcmc.il5, dmp.mcmc, x.new.il5 )
lm_rsl_cyt_op(il5_dmp_lmer$lm)


print("Summary for IL-5 and Total Diethyl Metabolites: ")
il5_dep_lmer  <- lmer.cyt_op( mcmc.il5, dep.mcmc, x.new.il5 )
lm_rsl_cyt_op(il5_dep_lmer$lm)


#il6

print("Summary for IL-6 and Total Metabolites: ")
il6_dap_lmer  <- lmer.cyt_op( mcmc.il6, dap.mcmc, x.new.il6 )
lm_rsl_cyt_op(il6_dap_lmer$lm)

print("Summary for IL-6 and Total Dimethyl Metabolites: ")
il6_dmp_lmer  <- lmer.cyt_op( mcmc.il6, dmp.mcmc, x.new.il6 )
lm_rsl_cyt_op(il6_dmp_lmer$lm)


print("Summary for IL-6 and Total Diethyl Metabolites: ")
il6_dep_lmer  <- lmer.cyt_op( mcmc.il6, dep.mcmc, x.new.il6 )
lm_rsl_cyt_op(il6_dep_lmer$lm)


#il8

print("Summary for IL-8 and Total Metabolites: ")
il8_dap_lmer  <- lmer.cyt_op( mcmc.il8, dap.mcmc, x.new.il8 )
lm_rsl_cyt_op(il8_dap_lmer$lm)

print("Summary for IL-8 and Total Dimethyl Metabolites: ")
il8_dmp_lmer  <- lmer.cyt_op( mcmc.il8, dmp.mcmc, x.new.il8 )
```

```
lm_rsl_cyt_op(il8_dmp_lmer$lm)


print("Summary for IL-8 and Total Diethyl Metabolites: ")
il8_dep_lmer  <- lmer.cyt_op( mcmc.il8, dep.mcmc, x.new.il8 )
lm_rsl_cyt_op(il8_dep_lmer$lm)


#il17

print("Summary for IL-17 and Total Metabolites: ")
il17_dap_lmer  <- lmer.cyt_op( mcmc.il17, dap.mcmc, x.new.il17 )
lm_rsl_cyt_op(il17_dap_lmer$lm)

print("Summary for IL-17 and Total Dimethyl Metabolites: ")
il17_dmp_lmer  <- lmer.cyt_op( mcmc.il17, dmp.mcmc, x.new.il17 )
lm_rsl_cyt_op(il17_dmp_lmer$lm)


print("Summary for IL-17 and Total Diethyl Metabolites: ")
il17_dep_lmer  <- lmer.cyt_op( mcmc.il17, dep.mcmc, x.new.il17 )
lm_rsl_cyt_op(il17_dep_lmer$lm)


#ifn

print("Summary for IFNg and Total Metabolites: ")
ifn_dap_lmer  <- lmer.cyt_op( mcmc.ifn, dap.mcmc, x.new.ifn )
lm_rsl_cyt_op(ifn_dap_lmer$lm)

print("Summary for IFNg and Total Dimethyl Metabolites: ")
ifn_dmp_lmer  <- lmer.cyt_op( mcmc.ifn, dmp.mcmc, x.new.ifn )
lm_rsl_cyt_op(ifn_dmp_lmer$lm)


print("Summary for IFNg and Total Diethyl Metabolites: ")
ifn_dep_lmer  <- lmer.cyt_op( mcmc.ifn, dep.mcmc, x.new.ifn )
lm_rsl_cyt_op(ifn_dep_lmer$lm)


#mcp

print("Summary for MCP and Total Metabolites: ")
mcp_dap_lmer  <- lmer.cyt_op( mcmc.mcp, dap.mcmc, x.new.mcp )
lm_rsl_cyt_op(mcp_dap_lmer$lm)
```

```
print("Summary for MCP and Total Dimethyl Metabolites: ")
mcp_dmp_lmer  <- lmer.cyt_op( mcmc.mcp, dmp.mcmc, x.new.mcp )
lm_rsl_cyt_op(mcp_dmp_lmer$lm)


print("Summary for MCP and Total Diethyl Metabolites: ")
mcp_dep_lmer  <- lmer.cyt_op( mcmc.mcp, dep.mcmc, x.new.mcp )
lm_rsl_cyt_op(mcp_dep_lmer$lm)


#tnf

print("Summary for TNF and Total Metabolites: ")
tnf_dap_lmer  <- lmer.cyt_op( mcmc.tnf, dap.mcmc, x.new.tnf )
lm_rsl_cyt_op(tnf_dap_lmer$lm)

print("Summary for TNF and Total Dimethyl Metabolites: ")
tnf_dmp_lmer  <- lmer.cyt_op( mcmc.tnf, dmp.mcmc, x.new.tnf )
lm_rsl_cyt_op(tnf_dmp_lmer$lm)


print("Summary for TNF and Total Diethyl Metabolites: ")
tnf_dep_lmer  <- lmer.cyt_op( mcmc.tnf, dep.mcmc, x.new.tnf )
lm_rsl_cyt_op(tnf_dep_lmer$lm)


```



```{r Redo regressions without season}
#logistic regression of respiratory endpoint on dimethyl dose term
print("Summary for Total Dialkyl Metabolites: ")
dap.lgstc<- glm.lgstc_op( dap.mcmc, x.new.il1 )
glm_rsl_op( dap.lgstc$glm )


print("Research Question 2: Are dialkyl OP metabolite levels associated with plasma cytokine
levels for children in the lower Yakima Valley?")

#il1
print("Summary for IL-1 and Total Dialkyl Metabolites: ")
il1_dap_lmer  <- lmer.cyt_op( mcmc.il1, dap.mcmc, x.new.il1 )
lmer_rsl_cyt_op(il1_dap_lmer$lm)

print("Summary for IL-1 and Total Dimethyl Metabolites: ")
```

```
il1_dmp_lmer  <- lmer.cyt_op( mcmc.il1, dmp.mcmc, x.new.il1 )
lmer_rsl_cyt_op(il1_dmp_lmer$lm)


print("Summary for IL-1 and Total Diethyl Metabolites: ")
il1_dep_lmer  <- lmer.cyt_op( mcmc.il1, dep.mcmc, x.new.il1 )
lmer_rsl_cyt_op(il1_dep_lmer$lm)

#il5
print("Summary for IL-5 and Total Dialkyl Metabolites: ")
il5_dap_lmer  <- lmer.cyt_op( mcmc.il5, dap.mcmc, x.new.il5 )
lmer_rsl_cyt_op(il5_dap_lmer$lm)

print("Summary for IL-5 and Total Dimethyl Metabolites: ")
il5_dmp_lmer  <- lmer.cyt_op( mcmc.il5, dmp.mcmc, x.new.il5 )
lmer_rsl_cyt_op(il5_dmp_lmer$lm)


print("Summary for IL-5 and Total Diethyl Metabolites: ")
il5_dep_lmer  <- lmer.cyt_op( mcmc.il5, dep.mcmc, x.new.il5 )
lmer_rsl_cyt_op(il5_dep_lmer$lm)

#il6
print("Summary for IL-6 and Total Dialkyl Metabolites: ")
il6_dap_lmer  <- lmer.cyt_op( mcmc.il6, dap.mcmc, x.new.il6 )
lmer_rsl_cyt_op(il6_dap_lmer$lm)

print("Summary for IL-6 and Total Dimethyl Metabolites: ")
il6_dmp_lmer  <- lmer.cyt_op( mcmc.il6, dmp.mcmc, x.new.il6 )
lmer_rsl_cyt_op(il6_dmp_lmer$lm)


print("Summary for IL-6 and Total Diethyl Metabolites: ")
il6_dep_lmer  <- lmer.cyt_op( mcmc.il6, dep.mcmc, x.new.il6 )
lmer_rsl_cyt_op(il6_dep_lmer$lm)

#il8
print("Summary for IL-8 and Total Dialkyl Metabolites: ")
il8_dap_lmer  <- lmer.cyt_op( mcmc.il8, dap.mcmc, x.new.il8 )
lmer_rsl_cyt_op(il8_dap_lmer$lm)

print("Summary for IL-8 and Total Dimethyl Metabolites: ")
il8_dmp_lmer  <- lmer.cyt_op( mcmc.il8, dmp.mcmc, x.new.il8 )
lmer_rsl_cyt_op(il8_dmp_lmer$lm)
```

```
print("Summary for IL-8 and Total Diethyl Metabolites: ")
il8_dep_lmer  <- lmer.cyt_op( mcmc.il8, dep.mcmc, x.new.il8 )
lmer_rsl_cyt_op(il8_dep_lmer$lm)


#il17
print("Summary for IL-17 and Total Dialkyl Metabolites: ")
il17_dap_lmer  <- lmer.cyt_op( mcmc.il17, dap.mcmc, x.new.il17 )
lmer_rsl_cyt_op(il17_dap_lmer$lm)

print("Summary for IL-17 and Total Dimethyl Metabolites: ")
il17_dmp_lmer  <- lmer.cyt_op( mcmc.il17, dmp.mcmc, x.new.il17 )
lmer_rsl_cyt_op(il17_dmp_lmer$lm)


print("Summary for IL-17 and Total Diethyl Metabolites: ")
il17_dep_lmer  <- lmer.cyt_op( mcmc.il17, dep.mcmc, x.new.il17 )
lmer_rsl_cyt_op(il17_dep_lmer$lm)


#ifn
print("Summary for IFN-gamma and Total Dialkyl Metabolites: ")
ifn_dap_lmer  <- lmer.cyt_op( mcmc.ifn, dap.mcmc, x.new.ifn )
lmer_rsl_cyt_op(ifn_dap_lmer$lm)

print("Summary for IFN-gamma and Total Dimethyl Metabolites: ")
ifn_dmp_lmer  <- lmer.cyt_op( mcmc.ifn, dmp.mcmc, x.new.ifn )
lmer_rsl_cyt_op(ifn_dmp_lmer$lm)


print("Summary for IFN-gamma and Total Diethyl Metabolites: ")
ifn_dep_lmer  <- lmer.cyt_op( mcmc.ifn, dep.mcmc, x.new.ifn )
lmer_rsl_cyt_op(ifn_dep_lmer$lm)

#mcp
print("Summary for MCP-1 and Total Dialkyl Metabolites: ")
mcp_dap_lmer  <- lmer.cyt_op( mcmc.mcp, dap.mcmc, x.new.mcp )
lmer_rsl_cyt_op(mcp_dap_lmer$lm)

print("Summary for MCP-1 and Total Dimethyl Metabolites: ")
mcp_dmp_lmer  <- lmer.cyt_op( mcmc.mcp, dmp.mcmc, x.new.mcp )
lmer_rsl_cyt_op(mcp_dmp_lmer$lm)


print("Summary for MCP-1 and Total Diethyl Metabolites: ")
mcp_dep_lmer  <- lmer.cyt_op( mcmc.mcp, dep.mcmc, x.new.mcp )
```

```
lmer_rsl_cyt_op(mcp_dep_lmer$lm)

#tnf
print("Summary for TNF-alpha and Total Dialkyl Metabolites: ")
tnf_dap_lmer  <- lmer.cyt_op( mcmc.tnf, dap.mcmc, x.new.tnf )
lmer_rsl_cyt_op(tnf_dap_lmer$lm)

print("Summary for TNF-alpha and Total Dimethyl Metabolites: ")
tnf_dmp_lmer  <- lmer.cyt_op( mcmc.tnf, dmp.mcmc, x.new.tnf )
lmer_rsl_cyt_op(tnf_dmp_lmer$lm)


print("Summary for TNF-alpha and Total Diethyl Metabolites: ")
tnf_dep_lmer  <- lmer.cyt_op( mcmc.tnf, dep.mcmc, x.new.tnf )
lmer_rsl_cyt_op(tnf_dep_lmer$lm)

```