THESIS

CELLULAR RESPONSES OF IMMORTALIZED HUMAN MONOCYTES FOLLOWING EXPOSURE TO AGRICULTURAL DAIRY PARTICULATE MATTER

Submitted by

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ABSTRACT

CELLULAR RESPONSES OF IMMORTALIZED HUMAN MONOCYTES FOLLOWING EXPOSURE TO AGRICULTURAL DAIRY PARTICULATE MATTER

Background: Agricultural particulate matter less than 10 μm in aerodynamic diameter (PM_{10}) is recognized as a contributor to respiratory and neurodegenerative diseases. Specifically, higher rates of exposure are known during agricultural practices. Still, the mechanisms linking PM exposure to airway inflammation remain incomplete or poorly defined. Lipid mediators such as prostaglandins and leukotrienes play a central role in inflammatory signaling, specifically in the respiratory system. Understanding their signals may clarify how agricultural PM contributes to both local and systemic health effects.

Methods: Particulate matter was collected from inside parlor of a modern dairy farm in Fort Collins, CO, and used to expose THP-1 non-differentiated human monocytes at 1, 10, and 100 mg/mL for 6, 12, and 24 hours. Eicosanoid production and expression of key biosynthetic enzymes were assessed by ELISA and real-time PCR. RNA transcripts for eicosanoid-regulating enzymes were quantified alongside secreted lipid mediators. Viability assays established non-lethal exposure conditions appropriate for inflammatory assessment.

Results: Dose- dependent increases in prostaglandin E_2 (PGE_2) of 100-200 pg/ml and to a lesser extent leukotriene E_4 (LTE_4) increased by around 25 pg/ml, but remained relatively stable. These changes were accompanied by a 10x up-regulation of cyclooxygenase-2 (COX-2)

and a 2x upregulation of arachidonate 15-lipoxygenase (ALOX-5), though it still was stable in its regulation by dose and times used. The results were collected while maintaining greater than 70% viability within the dose and time groups.

Conclusions: This study will provide mechanistic insight into how agricultural PM activates inflammatory pathways including eicosanoid production in both immune and epithelial cells. This research will advance understanding of PM-induced airway disease, including asthma, COPD, and allergic rhinitis, while also suggesting potential for systemic effects.

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CHAPTER 1: LITERATURE REVIEW

Overview of Particulate Matter Exposure 1.1:

You cannot exist in the world today without being exposed to particulate matter. Particulate matter refers to solid or liquid particles suspended in air that can typically be grouped into particles greater than 10 micrometers in diameter, referred to as an inhalable fraction, and those that are smaller than 10 micrometers in diameter, the respirable fraction (Deng et al., 2019a; Schaeffer et al., 2017). Particulates have a range of sources, including vehicle emissions, industrial processes, construction, wildfires, and agriculture. The constituents and size fractions will be different based on these sources. Particulate matter enters the body when inhaled; the effects change depending on how far the particulate reaches the respiratory system based on aerodynamic diameter and other physiological factors (e.g., breathing rate). These effects are due to differing cells lines of the respiratory system and the ability of various size fractions of PM to be filtered by the structure of the bronchial, which stops larger PM from making its way into deep alveolar space. PM 10 can carry causes reactive oxygen species (ROS) depending sources (PAHs, transition metals, incomplete combustion compounds) and may cause ROS from the stimulation and damage of immune cells, leading to pro-inflammatory lipid mediators (Arias-Pérez et al., 2019; Chen et al., 2022; Dennis & Norris, 2015; Kolmert et al., 2018; Lin et al., 2024; Radmark, 2022). These responses from the body can cause systemic effects by means of

infiltration and extrusion, and in cases of small enough particulates (PM < 2.5) diffusion via translocation of these particles (Deng et al., 2019b; Peters et al., 2006). These responses from the body can cause systemic effects that consequently affect the cardiovascular, respiratory, nervous, and reproductive systems. These symptoms are well documented for sources like traffic-related air particulates and urban dust (Arias-Pérez et al., 2019; Dockery et al., 1993). However, less research has been conducted on how agricultural dust least to the production of pro-inflammatory lipid mediators, downstream impacts, and systemic inflammation. Understanding sources is essential for many populations as there is an increase in communities that are moving closer to large scale farming operations and rising numbers of wildfires (McClure & Jaffe, 2018).

Inflammatory signaling 1.2:

Particulate matter has different effects depending on chemical composition and size caused by different processes occurring like resuspension by animal movement animal pens or combustion reactions from vehicles or fires. Inflammation is the signaling and chemical responses of the body to fend of against exogenous toxins and toxicants or in response to damaged tissues. Long-term effects of inflammation include asthma, COPD, cardiovascular disease, cancer, and neurodegenerative conditions (Arias-Pérez et al., 2019; Davydow et al., 2026; Dockery et al., 1993; Gunawan et al., 2024; Li et al., 2019; Marescaux et al., 2016). By understanding differing biomarkers from particulate matter, better research can be done to understand how to understand more about the innate immune response and identifying potential therapeutic targets. By better understanding these biomarkers, public health professionals can better address the prevention, early detection, treatment, and monitoring of chronic diseases and environmental exposures. Some of the lesser studied biomarkers include a class of lipids known as eicosanoids like PGE_2 and LTE4 (Gómez et al., 2019; Kolmert et al., 2018). These

biomarkers are created from the breakdown of polyunsaturated fatty acids (PUFA) like arachidonic acid (AA). They up-regulate inflammatory cytokines and chemokines and are often used as indicators of inflammation (Dennis & Norris, 2015). This study can further elucidate the systemic health impacts of PM exposure via different dust sources. Dust exposure causes a circular inflammatory effect where the exposure causes inflammatory biomarkers like eicosanoids that will increase the production of cytokines and chemokines, which cause a further increased eicosanoid production and inflammation (Dennis & Norris, 2015). This cascading inflammation causes acute and chronic effects. (Borlee et al., 2017; Chen et al., 2022; Marescaux et al., 2016; Dockery et al., 1993)

Scope of Study 1.3:

This study aims to better characterize the effects of particulate matter exposure on THP-1 cells. Specifically, the scope of this study is to assess the impact on respiratory system inflammation and the potential for systemic inflammation. Respiratory inflammation represents the initial localized response to inhaled particulate matter within the lungs, whereas systemic inflammation develops when inflammatory mediators generated in the respiratory tract disseminate into the bloodstream and influence distant organs. It includes assorted studies investigating various sources of PM 10 and their differing effects on public health. These sources included agricultural dust, Urban pollution, wildfire, and smoke. The studies of most interest included those in agricultural settings, specifically those in dairy farms or similar animal husbandry operations. Critical reviews, in-vitro, and in-vivo studies are gathered and compiled based on the effects of PM 10 on regulating the acute and chronic response of respiratory cells in deeper unciliated parts of the respiratory pathway. These unciliated areas beyond the upper respiratory system are of interest as the particulates are not carried away via the mucociliary

escalator. These studies were then divided into categories based on the specific biomarkers they were looking for, with oxylipins pathways of particular interest, as well as the effects the biomarkers may have on different body systems.

Key Concepts 1.4

The body's upper respiratory defenses generally filter out the larger particles (PM_{10} and larger), while finer particles (PM_{10} and smaller) penetrate deeper into the lungs and even at sizes of less than 2.5 um and ultra fine particulate may enter into the bloodstream, posing significant health risks (Benka-Coker et al., 2019; Chen et al., 2022; De Grove et al., 2018; Reid et al., 2016). The degree of inflammatory response varies by size and constituents. However, the cascading effects of inflammation are still caused by the same biomarkers (i.e. cytokines, chemokines, reactive oxygen species, prostaglandins, and leukotrienes). Many of these biomarkers are non-specific, so they are fast-acting in response to exposure.

Historical Background 1.5

The combination of dust from soil disturbance, emissions from animal waste, chemical particulates from fertilizers, smoke from residue burning, and diesel exhaust can create a high concentration of both PM_{10} and $PM_{2.5}$ around modern mass farming operations. These particles can cause or worsen respiratory conditions (such as asthma and COPD), cardiovascular disease, and other systemic health problems, particularly for those who live or work close to these large farms (2016; Dockery et al., 1993; Borlee et al., 2017; Dennis & Norris, 2015; Marescaux et al., 2016).. These modernized farms are proving to be detrimental to the farmers and those near these operations as studies show that increasing rates of illness are occurring in these populations as compared to other populations.

Databases and Search Strategy 1.6

The literature search was conducted using Google Scholar and PubMed. Search terms included combinations of the following keywords: "LTE4," "PGE_2," "agricultural," "air pollution," "in vitro," "cognitive," "particulate matter," "neurological," "respiratory," "arachidonic acid metabolism," "inflammation," "wildfire smoke," "eicosanoids," "trends of particulate matter," and "oxylipins." The search primarily focused on studies published within the past ten years to ensure the inclusion of current and relevant findings, while earlier works were considered when necessary to provide historical context. Studies were screened in accord with predefined inclusion and exclusion criteria to ensure relevance to the particulate matter-induced inflammatory responses and oxylipin-mediated pathways. The criteria used for study selection are summarized in Table 1.

Table 1. Inclusion and exclusion criteria

Category	Inclusion Criteria	Exclusion Criteria
Biomarkers	Studies that examine acute inflammatory biomarkers involved in oxylipin regulation, including prostaglandins and leukotrienes.	Studies that did not evaluate acute inflammatory biomarkers related to oxylipin pathways.
Exposure and Response	Studies investigate immune or inflammatory responses associated with particulate matter (PM) exposure.	Studies unrelated to respiratory or systemic inflammatory responses resulting from PM exposure.
Biological Systems	Studies focusing on respiratory system responses and associated inflammatory mechanisms.	Studies outside the scope of respiratory or systemic inflammation
Study design	In vitro studies, in vivo studies, and critical review articles addressing	Studies lacking relevance to mechanistic or health outcome associations related to PM-induced inflammation.

	mechanistic pathways or health outcomes.	
Publication Characteristics	Peer-reviewed articles published in English	Articles not available in English or lacking peer review.

Review of Key Literature 1.7

The review is structured by the PM sources and the physiological effects. Sources of the PM include Urban environments, agricultural dust, and wildfires. These physiological systems are affected by these sources of PM range via entry to the respiratory system and entrance into the blood via alveolar spaces and ultra-fine particulate matter (UFPM) that may pass through the upper respiratory system. The scope of the study is on systemic inflammation and will focus on the effects on respiratory systems.

The main discourse points on this subject are based on how the respiratory system's acute response affects other body systems. There are two main lines of thought on this topic. These fine particulates either cause inflammation via the deep respiratory system, causing biomarkers that pass into the bloodstream and become systemic, or UFPM, which can enter the bloodstream via the nasal olfactory bulb in the upper respiratory system.

Air Pollution 1.8

The respiratory effects of PM exposure have been extensively characterized through epidemiological, toxicological, and clinical studies (2016; Dockery et al.; Benka-Coker et al., 2019; Borlee et al., 2017; Gunawan et al., 2024; Lin et al., 2024). The respiratory tract can be broadly divided into the upper airways, bronchial airways, and alveolar regions, with particle deposition largely determined by aerodynamic diameter (Deng et al., 2019a; Schaeffer et al.,

2017). Larger particles are typically deposited in the upper respiratory tract through impaction and sedimentation, whereas fine ($PM_{2.5}$) and ultrafine particles (<0.1 μm) can penetrate deeply into the alveolar regions of the lung. Because alveoli serve as the primary site of gas exchange and possesses a large surface area near the pulmonary circulation, deposited particles can initiate local inflammatory responses with the potential to extend beyond the respiratory system.

Upon deposition within the lower respiratory tract, particulate matter can induce oxidative stress and activate resident immune cells, including alveolar macrophages and epithelial cells. This activation promotes the release of pro-inflammatory cytokines, chemokines, and lipid mediators that contribute to pulmonary inflammation and may subsequently enter systemic circulation (Radmark, 2022). The resulting inflammatory cascade has been proposed as a key mechanism linking inhaled particulate matter to adverse health outcomes affecting multiple organ systems (Arias-Pérez et al., 2019; Deng et al., 2019c; Gunawan et al., 2024; Kilian & Kitazawa, 2018; Li et al., 2019). This pathway is a complex mosaic of multiple cell lines leading to different signaling pathways and mediators which leads to the systemic effect on various organ systems. Due to the complexity of these pathways and the interplay between these systems is why there is so much scientific data that would prove valuable to further our understanding through these studies.

Chronic exposures have been linked to the development and progression of chronic respiratory diseases, including COPD, chronic bronchitis, and lung cancer (Borlee et al., 2017; AMERICAN THORACIC SOCIETY, 1998; Dockery et al., 1993). Acute exposure to elevated concentrations of particulate matter has been associated with airway irritation, exacerbation of asthma, reduced pulmonary function, increased respiratory symptoms, and heightened

susceptibility to respiratory infections (Chen et al., 2022; Dennis & Norris, 2015). Common symptoms include coughing, wheezing, dyspnea, and chest discomfort. Collectively, these findings demonstrate that inhaled particulate matter represents a significant public health concern due to its ability to initiate both localized pulmonary injury and systemic inflammatory responses.

PM composition 1.9

Agricultural PM is a complex mixture of minerals, metals, and organic constituents, including dander, pollen, microorganisms and associated biologically active products (e.g., inflammagens). Agricultural environments contain large amounts of aerosolized microbial material (Reynolds et al., 2005; Schaeffer et al., 2017). One of the most important inflammagen present in agricultural dust is endotoxin, also known as lipopolysaccharide (LPS), a component of the outer membrane of Gram-negative bacteria. Due to this large endotoxin composition, inhaled PM can deliver endotoxin directly to airway epithelial cells and immune cells, where it promotes inflammatory signaling.

The inflammatory activity of LPS is largely attributed to 3-hydroxy fatty acids (3-OHFAs). These fatty acids are commonly measured as chemical markers of endotoxin in environmental dust samples because they provide an estimate of total LPS burden (Poole et al., 2010; Reynolds et al., 2005). Variation in 3-OHFA and chain length may also reflect differences in the bacterial composition of agricultural dust and may influence the inflammatory potency of the endotoxin present (Reynolds et al., 2005).

Once inhaled, LPS is recognized primarily through the TLR4 signaling pathway (Lu et al., 2008). LPS is transferred by lipopolysaccharide-binding protein and TLR4 receptor complex,

which activates downstream NF-κB and MAPK signaling pathways. This signaling induces the expression of inflammatory cytokines and enzymes such as COX-2 and ALOX-5. Endotoxin present in agricultural PM links inhaled dust exposure to the activation of inflammatory pathways relevant to respiratory and systemic health effects.

Neurological Systems Effects 1.10

Recent epidemiological and experimental studies suggest that agricultural workers may be at increased risk of adverse neurological outcomes from chronic exposure to airborne contaminants (Davydow et al., 2026; Gunawan et al., 2024). While the mechanisms underlying these effects remain somewhat of a mystery, systemic inflammation has emerged as a potential contributor to neuroinflammatory processes. Inhalation of agricultural particulate matter can stimulate pulmonary immune responses, leading to the release of pro-inflammatory cytokines and lipid mediators into the systemic circulation. Chronic elevations in these cytokines, chemokines, and ROS may compromise blood-brain barrier integrity, increase its permeability, and facilitate the entry of circulating mediators into the central nervous system. Once within the brain's environment, these signals can activate astrocytes and microglia, leading to neuroinflammation, oxidative stress, and disruption of neuronal homeostasis (Davydow et al., 2026). Consequently, understanding the eicosanoid pathways triggered by agricultural particulate exposure may provide important insights into the potential connection between occupational exposures and the risk of neurodegenerative disease.

PM's effects on physiology cause acute oxidative stress and a storm of pro-inflammatory biomarkers that may become systematic depending on where the particulates are deposited. This damage to neurons and increased permeability of the blood-brain barrier (BBB) cause increased toxicants in the brain (Davydow et al., 2026; Gimeno-Ferrer et al., 2026). These pro-

inflammatory effects cause chronic diseases such as Alzheimer's, Parkinson's, and other forms of neurodegenerative decline (Davydow et al., 2026). The way in which these diseases occur is still being studied intently, but the hypothesis is that this inflammation causes a weakened brain barrier, microglial activation, and neuronal damage.

THP-1 Cell Line 1.11

The THP-1 cell line is from FAB-M5 (acute monocytic leukemia), THP-1 cells are a widely used human monocytic leukemia cell line originally derived from the peripheral blood of male patients with acute monocytic leukemia. This line has become a standard in vitro model for studying innate immune and inflammatory responses. These cells are of human origin, have reproducible growth characteristics, and the capacity to differentiate into macrophage-like cells following stimulation with agents such as phorbol 12-myristate 13-acetate (PMA). As such, THP-1 cells provide a valuable alternative to primary monocytes for toxicology and immunology investigations as they offer reproducible and accessible models without variability between donors. These cells express numerous receptors, including Toll-like receptors (TLRs), enabling them to respond to environmental contaminants, such as PM, by activating inflammatory signaling pathways. Upon exposure to PM, THP-1 monocytes and macrophages have been shown to produce reactive oxygen species (ROS), pro-inflammatory cytokines, and lipid mediators associated with oxidative stress and systemic inflammation (Nath Neerukonda et al., 2018; Radmark, 2022). Their sensitivity to airborne contaminants, along with their ability to model human monocytic immune responses, makes THP-1 cells particularly useful for evaluating cellular and molecular mechanisms of PM-induced inflammation and its potential contribution to respiratory, neurological, and other systemic disease processes.

Cyclooxygenase-2 and Prostaglandin E_2 1.12

COX-2, is an inducible enzyme involved in the conversion of arachidonic acid into prostaglandin H_2, the precursor of several prostanoids, including PGE_2. COX-2 expression is rapidly upregulated in response to inflammatory stimuli, oxidative stress, cytokines, and environmental exposures (Lin et al., 2024). Elevated COX-2 activity results in increased production of PGE_2, a lipid mediator that regulates vasodilation, vascular permeability, pain signaling, and immune cell activation like macrophages, dendritic cells, and T-cells (Kalinski, 2012). PGE_2 has been implicated in numerous respiratory inflammatory conditions, including asthma, COPD, and particulate matter-induced pulmonary inflammation.

Particulate matter exposure has been shown to induce COX-2 expression through oxidative stress and activation of pro-inflammatory signaling pathways. Increased PGE_2 production following PM exposure may contribute to both local respiratory inflammation and systemic inflammatory responses, making COX-2 and PGE_2 relevant biomarkers for assessing cellular responses to agricultural dust exposure.

Arachidonate 5-Lipoxygenase and Leukotriene E_4 1.13

ALOX-5 catalyzes the initial steps of leukotriene biosynthesis by converting arachidonic acid into leukotriene A_4. Subsequent enzymatic reactions generate the cysteinyl leukotrienes LTC_4, LTD_4, and LTE_4 (Calde, 2020). Among these metabolites, LTE_4 is the most stable end-product and is commonly measured as an indicator of leukotriene pathway activation. Cysteinyl leukotrienes are potent mediators of inflammation, promoting leukocyte recruitment, bronchoconstriction, mucus secretion, and increased vascular permeability (Benka-Coker et al., 2019).

Activation of the ALOX-5 pathway has been observed following exposure to airborne pollutants and particulate matter. Inflammatory cells, including monocytes and macrophages, can increase leukotriene production in response to oxidative and inflammatory stimuli. Because LTE_4 reflects downstream activation of the leukotriene pathway, measurement of both ALOX-5 gene expression and LTE_4 production provides insight into inflammatory signaling associated with particulate matter exposure.

Synthesis and Critique 1.14

While there is evidence linking particulate matter exposure to adverse respiratory and systemic health effects, several limitations remain in the current literature. One major challenge is the composition of particulate matter sources. The focus of the study is on agricultural dust and the workers and populations that are at risk of exposure to this particulate. The intent is to protect and improve the health of those exposed. In contrast to agricultural PM, wildfire smoke, urban air pollution, and industrial emissions differ in their physical and chemical characteristics, making it difficult to compare studies directly. Also, many studies rely on environmental monitoring data and epidemiological associations, which provide useful information on health outcomes but often lack mechanistic insight into the cellular pathways underlying them.

While investigations have demonstrated that particulate matter exposure induces inflammatory responses, fewer studies have examined the role of eicosanoid signaling pathways, particularly the regulation of cyclooxygenase-2 (COX-2), arachidonate 5-lipoxygenase (ALOX-5), and their downstream lipid mediators, prostaglandin E_2 (PGE_2) and leukotriene E_4 (LTE_4). Existing studies frequently focus on broader inflammatory markers such as cytokines and

oxidative stress indicators, leaving the contribution of lipid-mediated inflammatory signaling less well characterized.

Limited research has examined these pathways in monocyte cell models following exposure to agricultural particulate matter. As a result, knowledge gaps remain regarding the mechanisms by which agricultural dust influences inflammatory lipid production, as well as the potential roles of COX-2 and ALOX-5 in mediating these responses. Addressing these gaps will improve understanding of the biological processes linking agricultural particulate matter exposure to respiratory and systemic inflammation.

Implications for Research 1.15

Understanding the source-specific effects of PM on systemic inflammation and related biomarkers will be instrumental in closing critical gaps in public health research. This knowledge will support the development of precise diagnostic tools, targeted interventions, and regulations tailored to different sources of PM exposure. Through such advancements, public health initiatives will be better equipped to mitigate the health impacts of PM and protect at-risk populations from chronic diseases linked to systemic inflammation.

The effects of particulate matter have recently been evolving as new evidence shows that a wide range of pro-inflammatory eicosanoids play a role in systemic inflammation and neurological diseases. Eicosanoids, specifically Prostaglandins (PGs) and leukotrienes (LTs) have been subject to research in various in vivo and vitro models looking at the effects PM has on differing cell lines. These biomarkers play a role in the innate immune response of monocytes to particulate matter. These effects have a broad range of physiological effects, including promoting or resolving inflammation, regulating immune cells, and responding to fever.

Understanding the impact of PM on a broader scale allows a focused approach to specific biomarkers while understanding the complexity of the innate immune response caused by PM.

Summary of Findings 1.16

There is agreement that the effects of air pollutants have increased mortality among those exposed for long periods (Dockery et al., 1993; Kilian & Kitazawa, 2018). The lesser-understood aspects concern how distinct types of air pollutant sources have differing effects based on their composition. Agricultural dust does not consist of the same constituents as urban dust. The systems they affect seem to be consistent among all types of particulate matter. They share the same mechanisms of action, leading to inflammatory cascades, though to varying degrees. This is affected by the time exposed to these PMs and underscores the value of studying occupational health, where exposures are consistent and may persist for an entire lifetime, depending on the type of work.

This study aims to elucidate these effects by exploring the effects of agricultural dust sources on human monocytes. These biomarkers can give understanding to what effect specific inflammatory mediators may have on the function of the respiratory system as well as downstream systemic effects on brain tissue as well as how neuronal degeneration may occur.

The effects that agricultural dust has on chronic respiratory issues and neural degeneration are not well tested, although diseases like COPD, allergic rhinitis, Alzheimer's disease, and Parkinson's are occurring in farmers at a higher rate than in other occupations (Chari et al., 2018; Petit et al., 2024; AMERICAN THORACIC SOCIETY 1998). Because of this difference, it would be possible to distinguish which cellular stress signals may be a direct cause of similar diseases associated with urban occupations. Further understanding of this

disease's pathogenesis will help pave the way for more research and better protection of populations exposed to these conditions.

Central question 1.17

How does PM-induced inflammation in respiratory cells contribute to lipid-mediated systemic inflammation, which may lead to downstream effects on the central nervous system (CNS)?

CHAPTER 2: INTRODUCTION

Particulate matter (PM) exposure remains a significant concern in public and occupational health, particularly in agricultural environments where workers and nearby communities are regularly exposed to high concentrations of airborne dust. However, there are various sources of PM that remains a focus in public health. Particulate matter (PM) originates from a variety of natural and human sources. Urban PM is primarily generated through vehicle emissions, industrial activities, fossil fuel combustion, and road dust resuspension. These particles often contain combustion byproducts, metals, and other pollutants associated with transportation and industrial processes. In contrast, agricultural PM is generated by farming activities such as livestock operations, feed handling, tilling, harvesting, and vehicle movement on unpaved roads. This PM is typically composed of soil particles, organic matter, microorganisms, endotoxins, pollen, and animal-derived materials. However, the composition varies across agricultural environments depending on the work being done. PM_{10} and ultra-fine PM below 2.5 μm generated during farming activities can penetrate the respiratory tract, triggering a cascade of biological responses extending beyond localized irritation (Gunawan et al., 2024; Marescaux et al., 2016; Nath Neerukonda et al., 2018). From a public health perspective, these processes hold wide-reaching implications. Agricultural PM is not confined to the workplace; it can disperse into surrounding communities(up to 5 km)(Schaeffer et al., 2017), elevating risks of asthma, chronic obstructive pulmonary disease (COPD), allergic rhinitis, and potentially systemic diseases (Borlee et al., 2017; Chari et al., 2018; Davydow et al., 2026; Marescaux et al., 2016). The intersection between air quality, chronic disease burden, and health

disparities underscores the importance of identifying and mitigating these exposures at the population level. Regulatory bodies understand the impact of PM10 and PM2.5.

Agricultural workers are at increased risk of encountering repetitive, prolonged exposures to higher concentrations (NORA AgFF Sector, 2018). This leads to an increase in susceptibility to airway inflammation, oxidative stress, and long-term respiratory decline (Borlee et al., 2017; Chari et al., 2018; Petit et al., 2024). Monitoring biological markers of exposure and inflammatory responses can provide insight for developing interventions which could include engineering controls, personal protective equipment, and workplace health policies. These perspectives highlight the urgent need to characterize how agricultural PM initiates and amplifies inflammatory pathways. Understanding these mechanisms is essential not only for protecting the health of farm workers but also for informing broader strategies of environmental, occupational, and overall public health.

Proinflammatory mediators, which can trigger a systemic cascade of inflammatory responses like ROS generation, cytokines, chemokines, and further recruitment of innate immune cells, are an important part of understanding what signals and markers are at play in the increased risk of illness observed in these agricultural communities (NORA AgFF Sector, 2018; Petit et al., 2024). Due to the expansive list of potential mechanisms at play, looking at understudied options and the mechanisms of which they are synthesized gives a springboard into important gaps of this research. Increasing evidence indicates that these exposures initiate pro-inflammatory signaling through the release of lipid mediators, such as prostaglandins and leukotrienes, which are regulated by cyclooxygenase (COX-2) and lipoxygenase (ALOX-5) enzymatic pathways.

Monocytes and macrophages play an essential role in the innate immune response to inhaled particulate matter. Following particle deposition in the respiratory system, innate inflammatory signaling cascades are activated. Activation of these monocytes and macrophages results in the production of cytokines, chemokines, reactive oxygen species, and pro-inflammatory lipid mediators, which contribute to local pulmonary inflammation and systemic inflammatory effects (De Grove et al., 2018; Deng et al., 2019b). Because of their critical role in initiating inflammatory responses, monocytes have become a widely used model for the biological effects of particulate matter exposure.

One class of inflammatory mediators of interest is the eicosanoids. These are a family of lipid signaling molecules derived from arachidonic acid. The COX and LOX pathways represent two branches of eicosanoid metabolism(Calde, 2020; Petit et al., 2024; Radmark, 2022). Cyclooxygenase-2 (COX-2) is an inducible enzyme expressed during inflammation. The purpose of COX-2 is to catalyze the formation of prostaglandins, including prostaglandin E2 (PGE_2). PGE_2 is a mediator involved in vasodilation, immune regulation, and inflammatory signaling. Inflammatory signaling is the communication that initiates inflammatory responses, while immune regulation refers to the mechanisms that adjust these signaling events to control the intensity and duration of the response. Similarly, ALOX-5 initiates leukotriene synthesis, leading to the formation of leukotriene E4 (LTE4), which is associated with leukocyte recruitment, bronchoconstriction, and airway inflammation. The regulation of these pathways has been implicated in inflammatory and respiratory diseases and may represent a critical mechanism by which agricultural particulate matter causes adverse health effects.

To explore these inflammatory pathways, we quantified changes in gene expression and mediator production using reverse transcription quantitative polymerase chain reaction (RT-

qPCR) to assess the transcriptional alterations in inflammatory genes, including COX-2 and ALOX-5. While RT-qPCR evaluates changes at the mRNA level, enzyme-linked immunosorbent assays (ELISAs) measure downstream protein products and inflammatory mediators. Together, these complementary approaches provide insight into both the transcript regulation and activation of inflammatory pathways.

In the present study, THP-1 monocytes were exposed to agricultural particulate matter at concentrations of 1, 10, and 100 μg/mL for 6, 12, and 24 hours to evaluate dose- and time-dependent inflammatory responses. These exposure conditions were designed to characterize both early and sustained cellular reactions to particulate matter. The expression of COX-2 and ALOX-5 transcripts was assessed using RT-qPCR as indicators of cyclooxygenase and lipoxygenase pathway activation, while the levels of the downstream lipid mediators prostaglandin E2 (PGE_2) and leukotriene E4 (LTE4) were quantified via ELISA. By examining changes in both gene expression and mediator production across various exposure durations and concentrations, this study aimed to determine whether agricultural particulate matter promotes inflammatory signaling through these oxylipin-associated pathways(Kolmert et al., 2018; Radmark, 2022).

Purpose 2.1:

The purpose of this study is to investigate the mechanistic pathways by which agricultural PM_{10} contributes to airway inflammation. Specifically, we aim to characterize the signaling of lipid mediators in two complementary human cell models: THP-1 derived macrophages, representing innate immune responses. We will quantify the production of key eicosanoids, including LTE_4 and PGE_2, and evaluate the expression of critical metabolic enzymes such as COX-2 and ALOX-5. By integrating outcomes across these endpoints, this

study seeks to elucidate how agricultural PM modulates both immune and epithelial signaling pathways that are central to the pathology of chronic respiratory diseases, including asthma, COPD, and allergic rhinitis. A deeper understanding of these molecular events may provide insight into the role of agricultural air pollution in occupational and environmental respiratory health, with implications for prevention, risk assessment, and policy.

Additionally, we sought to ascertain if PM_{10} exposure elicits dose- and time-dependent increases in pro-inflammatory eicosanoids (PGE_2 and LTE_4) and upregulation of key enzymes (COX-2, ALOX-5) in both THP-1 macrophages, implicating lipid mediator signaling as a mechanism of PM-induced airway inflammation. We hypothesize that exposure to agricultural PM_{10} induces a dose- and time-dependent increase in pro-inflammatory eicosanoids, specifically PGE_2 and LTE_4, in THP-1 monocytes. This response will be accompanied by upregulation of eicosanoid-synthesizing enzymes, including COX-2 and ALOX-5, thereby linking agricultural PM exposure to airway inflammation through complementary immune and epithelial pathways.

Scope 2.2

This study focuses on characterizing the inflammatory signaling induced by agricultural PM_{10} in human-relevant in vitro models. Specifically, we will evaluate the production of lipid mediators, including PGE_2 and LTE_4, to assess the expression of key biosynthetic enzymes such as COX-2 and ALOX-5 in THP-1 monocytes. By integrating immune and epithelial responses, this work aims to provide a mechanistic understanding of how agricultural PM initiates airway inflammation.

The scope of this study is limited to cellular models and molecular endpoints and does not include in vivo experiments or epidemiological analysis. While the findings are not intended

to directly quantify human risk, they will establish a mechanistic basis for understanding how agricultural dust exposure may contribute to respiratory conditions such as asthma, COPD, and allergic rhinitis. Ultimately, this work seeks to inform future research directions, including vivo validation and population-level assessments, and to contribute to public health and occupational health strategies for mitigating agricultural air pollution risks.

CHAPTER 3: METHODS AND MATERIALS

Particulate collection 3.1

PM was collected from a high-volume dairy operation in the High Plains and Intermountain region to represent agriculturally derived dust typical of rural production environments. These samples were collected and size fractionated using a high-volume cascade impactor deployed on site to differentiate the fine and coarse PM encountered by workers inside the dairy. The impactor operated for 72 hours at 1500 liters per minute in three dairies. Size-segregated PM was collected across four different aerodynamic size fractions (<3, 3−10, 10−30, and 30-100 μm) (Schaeffer et al., 2017). The initial three stages of the impactor were designed to capture particulate matter (PM) onto separate Teflon substrates, facilitating the recovery and processing of the samples. The PM undergo gamma irradiation using cesium-137 (3,000 rads) to inhibit the growth and reproduction of microorganisms; however, based on previous studies (Schaeffer et al., 2017), the PM will still retain the causative agents of inflammation, such as lipopolysaccharides from the broken cell walls of bacteria.

THP-1 monocytes were maintained in supplemented medium containing 10% FBS, 1 mM sodium pyruvate, and 1% penicillin–streptomycin at 37°C in a humidified atmosphere of 5% CO_2. PM samples irradiated, then suspended in phosphate-buffered saline (PBS) as the vehicle control, vortexed to ensure uniform dispersion, and administered to cells at final concentrations of 1, 10, and 100 μg/mL; PBS-only exposures served as unexposed controls.

THP-1 cells were exposed at densities ranging from 0.5–5 × 10^6 cells/mL. Cells were exposed for 6, 12, and 24 hours to assess acute inflammatory responses.

Cell viability 3.2

Cell viability following PM exposure was assessed using the Cell Titer-Blue (CTB) Cell Viability Assay (Thermo Fisher Scientific, Waltham, MA). THP-1 cells were seeded into 96-well plates at a density of approximately 5 × 10^4 cells per well and allowed to equilibrate prior to exposure. Cells were exposed to PM concentrations of 1, 10, and 100 µg/mL suspended in phosphate-buffered saline (PBS). Untreated cells served as negative controls, while hydrogen peroxide (H_2O_2) treatment was used as a positive cytotoxicity control. Following the exposure period, CTB reagent was added according to the manufacturer's instructions and plates were incubated at 37 °C and protected from light. Fluorescence was measured using a Versa Max microplate reader (Molecular Devices, San Jose, CA) at wavelengths of 590 nm. Cell viability was calculated relative to untreated control cells. Values of 70% or greater were deemed as indicating limited cytotoxic effects.

RNA Isolation and Quantitative RT-PCR Analyses 3.3

THP-1 Cells were seeded in non–tissue culture-treated 6-well plates at a final volume of 3 mL per well and exposed to size-fractionated agricultural dust following sterilization by ionizing radiation. Cells were exposed to dust at final concentrations of 1, 10, and 100 µg/mL for 6, 12, and 24 hours. Cell viability assays (Thermo Fisher Scientific, Waltham, MA) were performed following exposure to confirm that observed molecular changes were not attributable to cytotoxicity.

At the end of each exposure period, cells were centrifuged and washed twice with PBS to remove residual particulate matter and potential coloring which may affect SYBR green fluorescence. Total RNA was extracted using the Qiagen RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Cells were lysed in RLT buffer supplemented with β-mercaptoethanol (BME) to ensure RNA stabilization and denaturation of RNases. RNA concentration and purity were assessed with a NanoDrop Lite spectrophotometer (Thermo Fisher Scientific, Waltham, MA), with acceptable A260/A280 ratios ranging from 2.00–2.15 and RNA concentrations ranging from 40-100 ng/ul.

Primer sequences were designed using Primer-BLAST (NCBI, Bethesda, MD) and synthesized by Integrated DNA Technologies (IDT, Coralville, IA). Primers were evaluated for self-dimers and hetero-dimers using IDT OligoAnalyzer. Amplification efficiency was found using standard curves, and melt curve analysis confirmed single, gene-specific amplification products without evidence of primer-dimer formation. Complementary DNA (cDNA) was created from total RNA using the iScript synthesis kit (Bio-Rad Laboratories, Hercules, CA). Equal amounts of RNA (25 ng) were used for each reverse transcription reaction to ensure consistency among samples. Reverse transcription was performed using an iCycler Thermal Cycler (Bio-Rad Laboratories). Quantitative reverse transcription PCR (RT-qPCR) was performed using SYBR Green chemistry (Bio-Rad, Hercules, California) to evaluate gene expression of COX-2, ALOX-5, and β-actin (ACTB) as the housekeeping gene. Amplification specificity was confirmed by melt curve analysis for each target using the Light Cycler 480 (Roche Diagnostics, Indianapolis, IN). Absolute quantification was conducted, and relative gene expression was calculated using the comparative Cycle threshold (Ct) method with β-actin as the internal control. Ct values for COX-2 and ALOX-5 were normalized to the housekeeping gene β-

actin (ACTB) to account for variation in RNA input and cDNA efficiency. Gene expression changes were analyzed relative to vehicle-treated controls to determine dose- and time-dependent transcriptional responses to agricultural dust exposure. Fold-change values derived from the ΔΔCt analysis were analyzed using a two-way ANOVA to evaluate the effects of particulate matter concentration (1, 10, and 100 μg/mL) and exposure duration (6, 12, and 24 h). Statistical significance was defined as $p < 0.05$.

Quantification of inflammatory Lipids by ELISA 3.4

Extracellular concentrations of PGE_2 and LTE4 were quantified in cell culture supernatants using competitive enzyme-linked immunosorbent assay (ELISA) kits obtained from Cayman Chemical (Ann Arbor, MI, USA). Assays were performed according to manufacturer protocols with minor modifications as described below.

Following particulate matter exposure, cell culture supernatants were collected and centrifuged at 1,500 × g for 10 minutes at 4 °C to remove suspended cells and particulate debris. Clarified supernatants were aliquoted into sterile polypropylene microcentrifuge tubes and stored at −80 °C until analysis to minimize freeze-thaw cycles. Prior to assay preparation, all reagents were equilibrated to room temperature and prepared according to manufacturer instructions. Standards for each analyte were generated by serial dilution in assay buffer across the recommended concentration ranges supplied with each kit. Preliminary dilution optimization experiments were conducted to ensure experimental absorbance values remained within the linear range of the standard curves.

For both assays, standards, blanks, controls, and samples were added to goat anti-mouse IgG-coated 96-well plates supplied with each kit. Competitive binding reactions were initiated by adding solutions of the analyte-specific tracer and monoclonal antibody.

The PGE_2 assay used the PGE_2 Express ELISA format, which included orbital shaking for 90-120 minutes at room temperature. The LTE4 ELISA used a standard competitive ELISA incubation procedure, with overnight incubation at 4 °C for approximately 18 hours to maximize assay sensitivity and competitive equilibrium.

Following primary incubation, wells were aspirated and washed five times with wash buffer to remove unbound reagents. Ellman's reagent was subsequently added to each well for colorimetric development. Plates were incubated on an orbital shaker protected from light until adequate signal development was achieved. Absorbance was measured at 412 nm using a spectrophotometer.

Standard curves for both analytes were generated using four-parameter logistic (4-PL) nonlinear regression analysis in GraphPad Prism. Concentrations of unknown samples were interpolated from the corresponding standard curves and corrected for dilution factors prior to statistical analysis. Assay quality was evaluated through duplicate agreement, standard curve performance, and manufacturer-recommended quality control criteria. Statistical significance was defined as $p < 0.05$.

CHAPTER 4: RESULTS

Cell Viability 4.1

THP-1 cell viability following exposure to agricultural dairy particulate matter was assessed using the CellTiter-Blue assay after 6, 12, and 24 h exposures to concentrations of 1, 10, and 100 µg/mL (Figure 1). Cell viability remained high across all treatment groups, ranging from approximately 70% to 95% of the untreated control values.

No clear dose-dependent reduction in viability was observed across the exposure concentrations tested. Viability remained generally consistent across exposure durations, indicating limited cytotoxic effects associated with agricultural particulate matter exposure under the conditions evaluated. Although some variability was observed among treatment groups, all exposures retained viability levels above 70% of control values.

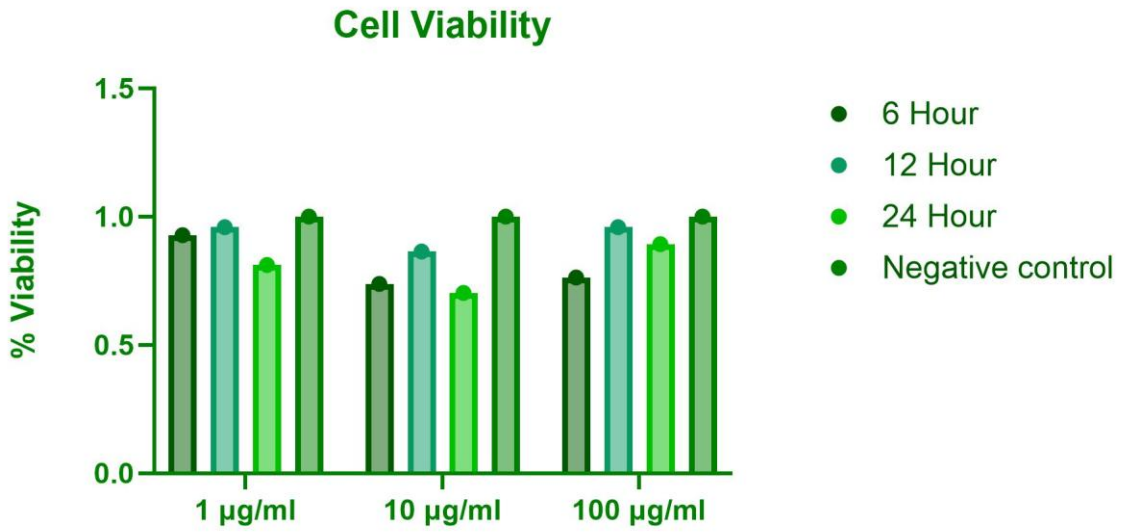
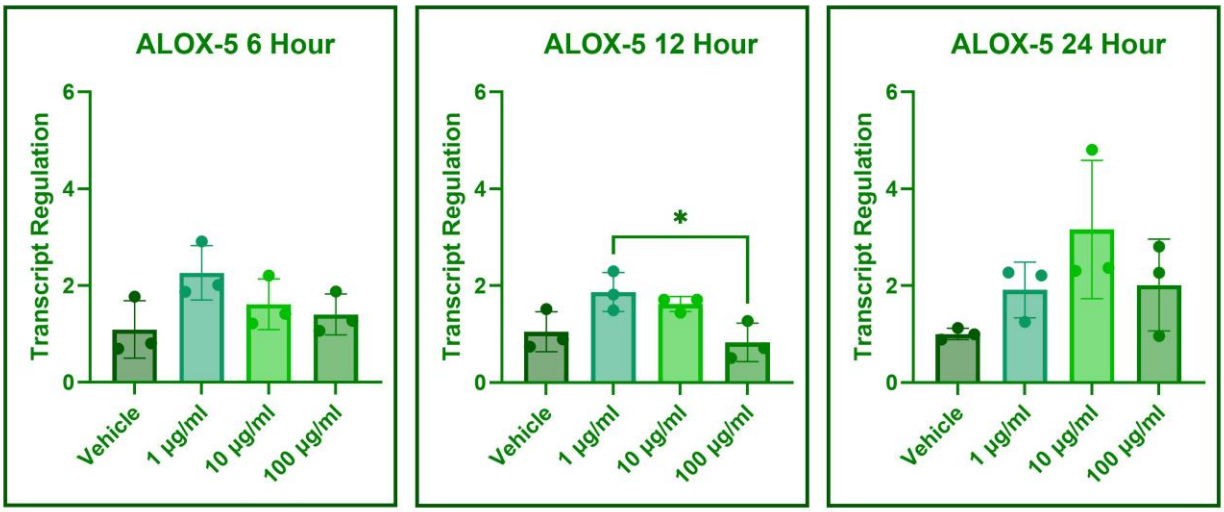


Figure 1. Cell Viability is unchanged following treatment.

THP-1 cells were exposed to agricultural dairy PM at concentrations of 1, 10, and 100 µg/mL for 6, 12, or 24 h. Cell viability was assessed using the CellTiter-Blue assay and expressed as a percentage of the untreated control and tested for significance.

PCR 4.2

Exposure of THP-1 monocytes to agricultural PM produced dose- and time-dependent changes in inflammatory gene expression (Figure 2). COX-2 expressions generally increased with higher PM concentrations across all exposure durations, with the most significant upregulation observed at 100 µg/mL following 6 and 24 hours of exposure when comparing to the vehicle ($p=0.0017$ and 0.0076, for 6 and 24 hours respectively). Although, these increases were consistent through the different replicates in upregulation from high dosing. In contrast, ALOX-5 expression exhibited a less consistent pattern, showing moderate induction at lower concentrations but a decrease at the highest concentration, suggesting a potential hormetic-like response.



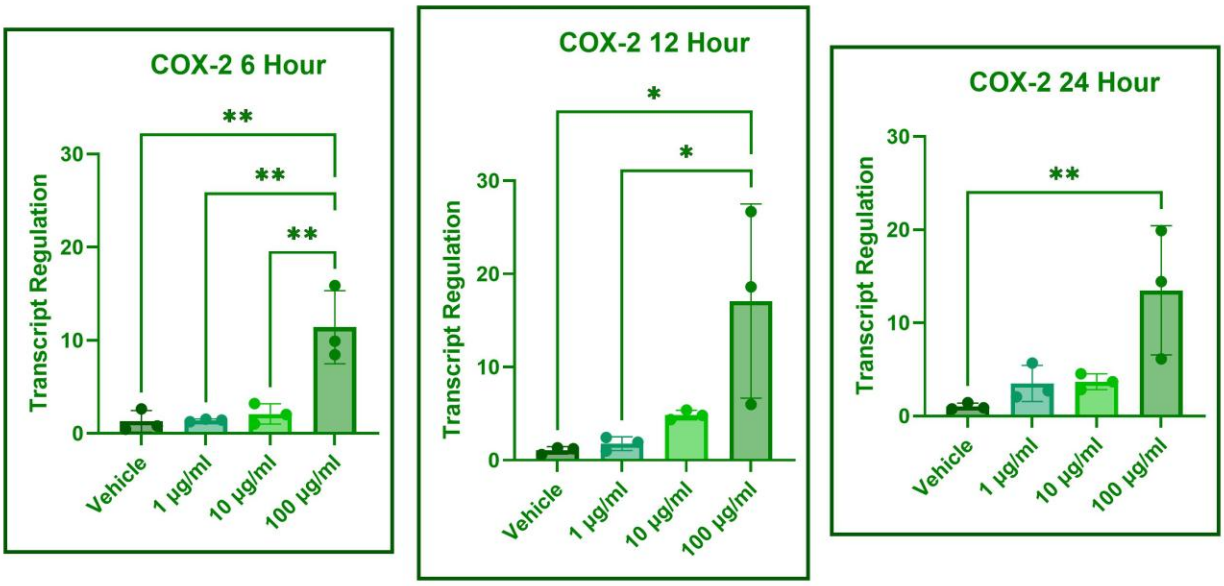


Figure 2. Significant increase in COX2 mRNA expression with PM_{10}

The relative expression of COX-2 and ALOX-5 in THP-1 monocytes grouped by time following particulate matter exposure. THP-1 monocytes were exposed to the agricultural particulate matter at 6, 12, and 24 hours at doses of 1, 10, and 100 ug/ml. Gene expression was quantified using SYBR Green RT-qPCR and normalized to ACTB using the DELTA-DELTA CT method relative to PBS, the vehicle control. Data is presented using a two-way ANOVA from three biological replicates with three technical replicates per sample.

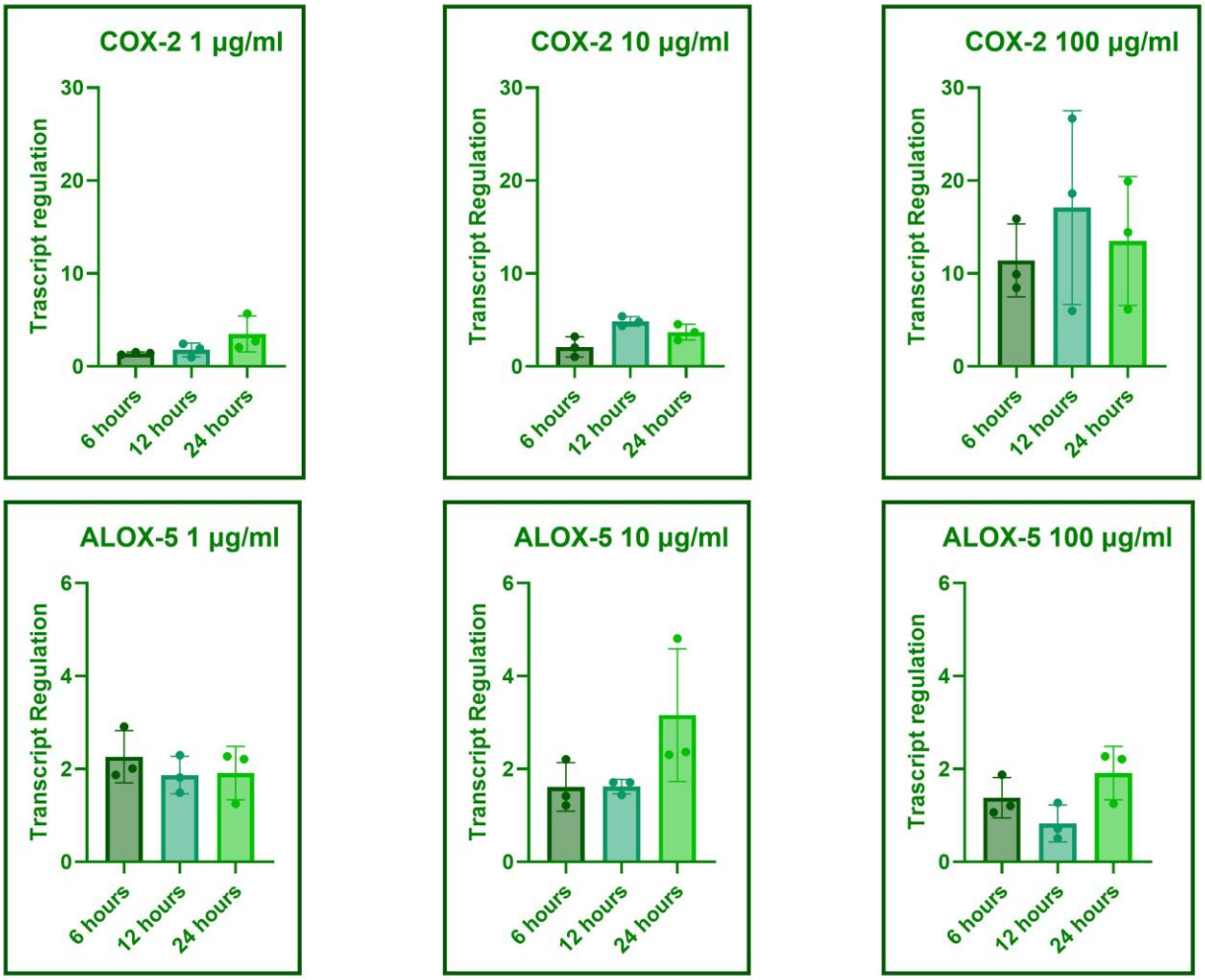


Figure 3. No significance within ALOX5 and COX-2 PCR grouped by dose

The relative expression of COX-2 and ALOX-5 in THP-1 monocytes grouped by dose following particulate matter exposure. THP-1 monocytes were exposed to the agricultural particulate matter at 6, 12, and 24 hours at doses of 1, 10, and 100 ug/ml. Gene expression was quantified using SYBR Green RT-qPCR and normalized to ACTB using the DELTA-DELTA CT method relative to PBS, the vehicle control. Data is presented using a two-way ANOVA from three biological replicates with three technical replicates per sample.

ELISA 4.3

Dose- and time-dependent increases in pro-inflammatory lipids were also observed in the THP-1 monocytes following exposure to agricultural PM (Figure 4). The PGE_2 expressions increased with higher PM concentrations across exposure durations, with the highest

concentration of the lipid observed after 6 hours of exposure to 100 µg/mL. The high dose for each time (6, 12, and 24) was significant. p = 0.0002, 0.0131, and 0.0002 respectively. Similar to the RT-qPCR results, the LTE4 exhibited a less consistent pattern, showing moderate up-regulation at lower concentrations but a decrease at the highest concentration for the 6- and 24-hour timing.

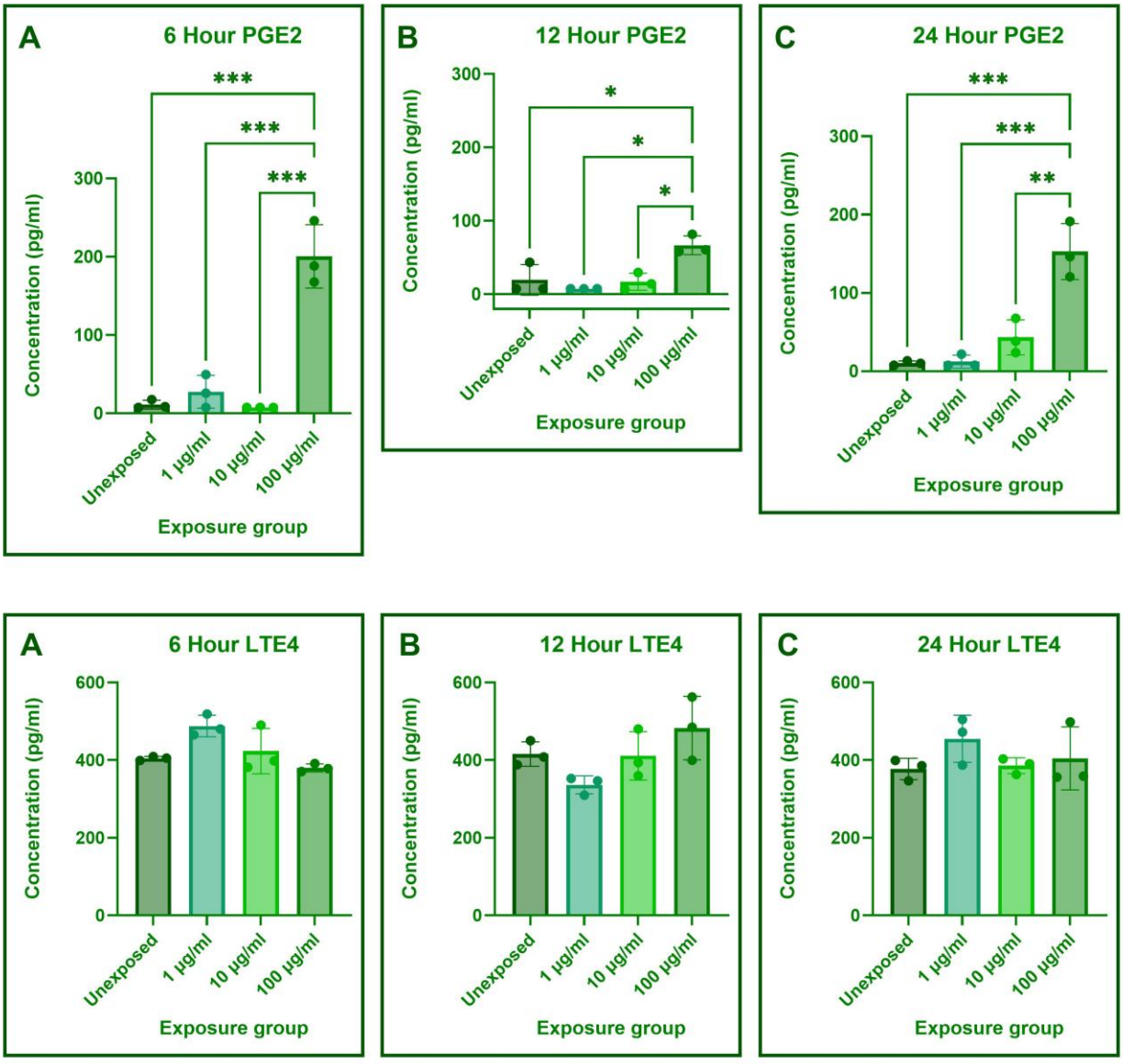


Figure 4. Significant increase in PGE_2 Presence After Exposure

Production of eicosanoid inflammatory mediators following agricultural particulate matter exposure in THP-1 monocytes. THP-1 monocytes were exposed to agricultural particulate matter at concentrations of 1, 10, and 100 μg/mL for 6, 12, and 24 h. Levels of PGE_2 and LTE4 were quantified using competitive ELISAs. Concentrations were determined from standard curves and are reported as pg/mL. Data are presented as a two-way ANOVA across three biological replicates, with three technical replicates per sample.

CHAPTER 5: DISCUSSION

Summary of major findings 5.1

Exposure of THP-1 monocytes to agricultural particulate matter resulted in alterations in inflammatory markers associated with the COX-2 and ALOX-5 pathways. Both exposure concentration and duration influenced gene expression, with COX-2 exhibiting a more pronounced dose- and time-dependent response than ALOX-5. These transcriptional changes were generally consistent with the ELISA findings, suggesting an association between gene expression and the production of the downstream lipid mediators PGE_2 and LTE4. However, the extent of mediator production did not always directly mirror changes in transcript expression, indicating the potential influence of post-transcriptional regulation and other cellular mechanisms. Compared to COX-2, ALOX-5 expression remained relatively stable across exposure conditions, which may partially explain the more limited changes observed in LTE4 production. Cell viability remained above 70% across all doses and time points, indicating that the observed molecular responses occurred in the absence of cytotoxicity.

Gene Expression Results 5.2

The gene expression results indicated differential regulation of the COX-2 and ALOX-5 pathways following exposure to agricultural particulate matter in THP-1 monocytes. Exposure increased COX-2 mRNA expression, with responses varying by dose and exposure duration. In contrast, ALOX-5 expression exhibited comparatively modest changes and showed limited evidence of a dose- or time-dependent response. These findings suggest that the COX-2 pathway may be more responsive to agricultural particulate matter exposure than the ALOX-5 pathway under these conditions. The relatively stable expression of ALOX-5 may indicate tighter regulatory control of the lipoxygenase pathway. These results suggest that agricultural particulate

matter preferentially activates inflammatory signaling associated with COX-2, while eliciting a weaker transcriptional response in the ALOX-5 pathway.

Interpretation of ELISA results 5.3

The competitive ELISA for PGE_2 revealed an increase in proinflammatory lipids with both dose and time. This observation aligned with the PCR results; however, discrepancies were noted between mRNA expression and mediator production at specific dosing intervals, particularly at 12 hours for high doses. Additionally, LTE4 exhibited consistent regulation, even among those exposed to the vehicle. These findings suggest that in these monocytes, the changes in the regulation of this eicosanoid is subtle and does not significantly alter following exposure (Haeggström, 2018; Haeggström & Funk, 2011).

Public Health Significance 5.4

Agricultural workers and residents of rural communities are regularly exposed to airborne particulate matter generated during livestock operations, feed handling, crop production, and other farming activities. Exposure to these particles has been associated with respiratory symptoms, reduced lung function, asthma exacerbation, chronic obstructive pulmonary disease (COPD), and systemic inflammatory effects. Understanding the biological mechanisms underlying these health outcomes is vital for developing effective exposure controls and public health interventions. By examining the upregulation of eicosanoid-synthesizing enzymes and the following eicosanoids by THP-1 monocytes following exposure to agricultural particulate matter, this study contributes to the growing body of evidence linking particulate exposure to immune activation. Specifically, evaluation of the COX-2/PGE_2 and ALOX-5/LTE_4 pathways may provide insight into how agricultural dust initiates inflammatory processes that could contribute to both respiratory and systemic disease. These findings may ultimately support

efforts to reduce occupational exposures, improve worker health protections, and inform environmental health policies to minimize the impacts of agricultural air pollution.

Study Limitations 5.5

Several limitations should be considered when interpreting these findings. First, exposures were conducted under submerged in vitro culture conditions rather than at an air–liquid interface (ALI), which more closely simulates the biological relevance and exposure of the human airway epithelium. Submerged exposure systems may alter particle deposition patterns and differentiation compared to physiologically relevant inhalation models. Second, the PM concentrations used in this study (1–100 μg/mL) were selected to evaluate dose–response relationships under controlled conditions but may not directly reflect deposited doses achieved in vivo following occupational or environmental inhalation. Extrapolation to human exposure scenarios should be interpreted with caution. Third, monoculture systems were used for THP-1 cells. This limits the ability to capture intercellular signaling and immune–epithelial crosstalk which could influence eicosanoid synthesis and inflammatory cascades. Co-culture airway models may better represent the coordinated cellular responses that occur in the respiratory tract. Finally, THP-1 cells are intrinsically from male donors which limits the generalizability of the study to those of a different sex(Ferretti, .; Shukla et al., 2021) Future studies incorporating ALI systems, male and female donors,.

CHAPTER 6: CONCLUSION AND FUTURE WORK

Conclusions 6.1

The objective of this study was to investigate the dose-dependent effects of agricultural PM exposure on the release of pro-inflammatory eicosanoids, specifically PGE_2 and LTE4, THP-1 monocytes. This study explored the impact of agricultural particulate matter on the regulation and response of THP-1 monocytes. The exposure led to an upregulation of COX-2 and ALOX-5 expression, as well as changes in the production of pro-inflammatory lipids such as LTE4 and PGE_2 . Notably, these results were obtained while ensuring cell viability remained at 70% or higher across all treatment groups. These findings indicate that agricultural PM triggers increased eicosanoid production in monocytes, irrespective of cytotoxicity or cell death. Collectively, these results enhance our understanding of agricultural PM's role in biological mechanisms contributing to respiratory and systemic inflammation.

Future Work 6.2

Building upon the findings of this study, there are opportunities for future work to enhance biological relevance. First, implementation of air–liquid interface (ALI) culture systems would allow for more physiologically relevant particulate exposure conditions that better mimic inhalation and airway deposition. ALI models would facilitate evaluation of particle–epithelial interactions under conditions that preserve epithelial differentiation, barrier integrity, and mucociliary function.

To integrate physiologically relevant doses into the study to approximate in vivo pulmonary deposition would help cement biological relevance. This is especially important when using an ALI model. This application of dosimetry models would assist in translating in vitro

concentrations to estimated deposited lung doses observed in occupational agricultural settings. This would improve the relevance of observed dose-dependent eicosanoid responses.

Incorporation of co-culture systems involving bronchial epithelial cells and monocytes would enable investigation of a more biologically relevant in vivo model that encompasses communication between immune and epithelial cells as a driver of the observed transcriptional and lipid mediator responses. Signaling between these cells types may amplify or modulate eicosanoid synthesis pathways, including COX-2 and ALOX-5 signaling, and better represent inflammatory cascades occurring in farmers or agricultural communities. Collectively, these approaches would strengthen mechanistic interpretation and improve the translational applicability of agricultural particulate exposure research to occupational respiratory health outcomes.

REFERENCES

Arias-Pérez, R. D., Taborda, N. A., Gómez, D. M., Fredy Narvaez, J., Porras, J., & Hernandez, J. C. (2019). Inflammatory effects of particulate matter air pollution. *Springer Nature*. https://doi.org/10.1007/s11356-020-10574-w/Published

Benka-Coker, W. O., Loftus, C., Karr, C., & Magzamen, S. (2019). Characterizing the joint effects of pesticide exposure and criteria ambient air pollutants on pediatric asthma morbidity in an agricultural community. *Environmental Epidemiology*, *3*(3). https://doi.org/10.1097/EE9.0000000000000046

Borlee, F., Joris Yzermans, C., Aalders, B., Rooijackers, J., Krop, E., Maassen, C. B. M., Schellevis, F., Brunekreef, B., Heederik, D., & Smit, L. A. M. (2017). Air pollution from livestock farms is associated with airway obstruction in neighboring residents. *American Journal of Respiratory and Critical Care Medicine*, *196*(9), 1152–1161. https://doi.org/10.1164/rccm.201701-0021OC

Calde, P. C. (2020). Eicosanoids. *Essays in Biochemistry*, *64*(3), 423–441. https://doi.org/10.1042/EBC20190083

Chari, R., Kress, A. M., & Madrigano, J. (2018). Injury and Illness Surveillance of U.S. Agricultural Workers. *Rand Health Quarterly*, *8*(2), 10.

Chen, H., Oliver, B. G., Pant, A., Olivera, A., Poronnik, P., Pollock, C. A., & Saad, S. (2022). Effects of air pollution on human health – Mechanistic evidence suggested by in vitro and in vivo modelling. *Environmental Research*, *212*. https://doi.org/10.1016/j.envres.2022.113378

Davydow, D. S., Pontone, G. M., Okun, M. S., Armstrong, M. J., Böttger, T. W., Geels, C., Frohn, L. M., Brandt, J., Dreier, J. W., Christensen, J., Pedersen, C. B., & Horsdal, H. T. (2026). Exposure to Air Pollutants and Lewy Body and Parkinson Disease–Related Dementias. *JAMA Network Open*, *9*(5), e2612601. https://doi.org/10.1001/jamanetworkopen.2026.12601

De Grove, K. C., Provoost, S., Brusselle, G. G., Joos, G. F., & Maes, T. (2018). Insights in particulate matter-induced allergic airway inflammation: Focus on the epithelium. *Clinical and Experimental Allergy*, *48*(7), 773–786. https://doi.org/10.1111/cea.13178

Deng, Q., Deng, L., Miao, Y., Guo, X., & Li, Y. (2019a). Particle deposition in the human lung: Health implications of particulate matter from different sources. *Environmental Research*, *169*, 237–245. https://doi.org/10.1016/j.envres.2018.11.014

Deng, Q., Deng, L., Miao, Y., Guo, X., & Li, Y. (2019b). Particle deposition in the human lung: Health implications of particulate matter from different sources. *Environmental Research*, *169*, 237–245. https://doi.org/10.1016/j.envres.2018.11.014

Deng, Q., Deng, L., Miao, Y., Guo, X., & Li, Y. (2019c). Particle deposition in the human lung: Health implications of particulate matter from different sources. *Environmental Research*, *169*, 237–245. https://doi.org/10.1016/j.envres.2018.11.014

Dennis, E. A., & Norris, P. C. (2015). Eicosanoid storm in infection and inflammation. *Nature Reviews Immunology*, *15*(8), 511–523. https://doi.org/10.1038/nri3859

Dockery, D. W., Pope, C. A., Xu, X., Spengler, J. D., Ware, J. H., Fay, M. E., Ferris, B. G., & Speizer, F. E. (1993). An Association between Air Pollution and Mortality in Six U.S. Cities. *New England Journal of Medicine*, *329*(24), 1753–1759. https://doi.org/10.1056/NEJM199312093292401

Ferretti, M. (n.d.). *Sex differences in Alzheimer disease—The gateway to precision medicine | Nature Reviews Neurology*. Retrieved June 5, 2026, from https://www.nature.com/articles/s41582-018-0032-9

Gimeno-Ferrer, F., Porschen, L. T., Matthes, F., Gohlsch, K., & Meissner, A. (2026). Airborne particulates and brain health: The role of PM2.5 in blood-brain-barrier dysfunction. *Journal of Cerebral Blood Flow & Metabolism*, *46*(5), 1025–1042. https://doi.org/10.1177/0271678X261418925

Gómez, C., Gonzalez-Riano, C., Barbas, C., Kolmert, J., Ryu, M. H., Carlsten, C., Dahlén, S. E., & Wheelock, C. E. (2019). Quantitative metabolic profiling of urinary eicosanoids for clinical phenotyping. *Journal of Lipid Research*, *60*(6), 1164–1173. https://doi.org/10.1194/jlr.D090571

Gunawan, C., Fleming, C., Irga, P. J., Jien Wong, R., Amal, R., Torpy, F. R., Mojtaba Golzan, S., & McGrath, K. C. (2024). Neurodegenerative effects of air pollutant Particles: Biological mechanisms implicated for Early-Onset Alzheimer's disease. *Environment International*, *185*. https://doi.org/10.1016/j.envint.2024.108512

Haeggström, J. Z. (2018). Leukotriene biosynthetic enzymes as therapeutic targets. *The Journal of Clinical Investigation*, *128*(7), 2680–2690. https://doi.org/10.1172/JCI97945

Haeggström, J. Z., & Funk, C. D. (2011). Lipoxygenase and Leukotriene Pathways: Biochemistry, Biology, and Roles in Disease. *Chemical Reviews*, *111*(10), 5866–5898. https://doi.org/10.1021/cr200246d

Kalinski, P. (2012). Regulation of Immune Responses by Prostaglandin E2. *Journal of Immunology (Baltimore, Md. : 1950)*, *188*(1), 21–28. https://doi.org/10.4049/jimmunol.1101029

Kilian, J., & Kitazawa, M. (2018). The emerging risk of exposure to air pollution on cognitive decline and Alzheimer's disease – Evidence from epidemiological and animal studies. *Biomedical Journal*, *41*(3), 141–162. https://doi.org/10.1016/j.bj.2018.06.001

Kolmert, J., Fauland, A., Fuchs, D., Säfholm, J., Gómez, C., Adner, M., Dahlén, S. E., & Wheelock, C. E. (2018). Lipid Mediator Quantification in Isolated Human and Guinea Pig Airways: An Expanded Approach for Respiratory Research. *Analytical Chemistry*, *90*(17), 10239–10248. https://doi.org/10.1021/acs.analchem.8b01651

Li, D., Zhang, R., Cui, L., Chu, C., Zhang, H., Sun, H., Luo, J., Zhou, L., Chen, L., Cui, J., Chen, S., Mai, B., Chen, S., Yu, J., Cai, Z., Zhang, J., Jiang, Y., Aschner, M., Chen, R., … Chen, W. (2019). Multiple organ injury in male C57BL/6J mice exposed to ambient particulate matter in a real-ambient PM exposure system in Shijiazhuang, China. *Environmental Pollution*, *248*, 874–887. https://doi.org/10.1016/j.envpol.2019.02.097

Lin, Y., Wang, X., Chen, R., Weil, T., Ge, Y., Stapleton, H. M., Bergin, M. H., & Zhang, J. "Jim." (2024). Arachidonic Acid Metabolites in Self-Collected Biospecimens following Campfire Exposure: Exploring Non-invasive Biomarkers of Wildfire Health Effects. *Environmental Science and Technology Letters*, *11*(3), 201–207. https://doi.org/10.1021/acs.estlett.3c00923

Lu, Y.-C., Yeh, W.-C., & Ohashi, P. S. (2008). LPS/TLR4 signal transduction pathway. *Cytokine*, *42*(2), 145–151. https://doi.org/10.1016/j.cyto.2008.01.006

Marescaux, A., Degano, B., Soumagne, T., Thaon, I., Laplante, J. J., & Dalphin, J. C. (2016). Impact of farm modernity on the prevalence of chronic obstructive pulmonary disease in Dairy farmers. *Occupational and Environmental Medicine*, *73*(2), 127–133. https://doi.org/10.1136/oemed-2014-102697

McClure, C. D., & Jaffe, D. A. (2018). US particulate matter air quality improves except in wildfire-prone areas. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(31), 7901–7906. https://doi.org/10.1073/pnas.1804353115

Nath Neerukonda, S., Mahadev-Bhat, S., Aylward, B., Johnson, C., Charavaryamath, C., & Arsenault, R. J. (2018). Kinome analyses of inflammatory responses to swine barn dust extract in human bronchial epithelial and monocyte cell lines. *Innate Immunity*, *24*(6), 366–381. https://doi.org/10.1177/1753425918792070

NORA AgFF Sector. (2018). *National Occupational Research Agenda for Agriculture, Forestry and Fishing May 2018*.

Peters, A., Veronesi, B., Calderón-Garcidueñas, L., Gehr, P., Chen, L. C., Geiser, M., Reed, W., Rothen-Rutishauser, B., Schürch, S., & Schulz, H. (2006). Translocation and potential neurological effects of fine and ultrafine particles a critical update. *Particle and Fibre Toxicology*, *3*(1), 13. https://doi.org/10.1186/1743-8977-3-13

Petit, P., Gondard, E., Gandon, G., Moreaud, O., Sauvée, M., & Bonneterre, V. (2024). Agricultural activities and risk of Alzheimer's disease: The TRACTOR project, a nationwide

retrospective cohort study. *European Journal of Epidemiology*, *39*(3), 271–287. https://doi.org/10.1007/s10654-023-01079-0

Poole, J. A., Dooley, G. P., Saito, R., Burrell, A. M., Bailey, K. L., Romberger, D. J., Mehaffy, J., & Reynolds, S. J. (2010). Muramic Acid, Endotoxin, 3-Hydroxy Fatty Acids, and Ergosterol Content Explain Monocyte and Epithelial Cell Inflammatory Responses to Agricultural Dusts. *Journal of Toxicology and Environmental Health. Part A*, *73*(10), 684–700. https://doi.org/10.1080/15287390903578539

Radmark, O. (2022). Formation of eicosanoids and other oxylipins in human macrophages. *Biochemical Pharmacology*, *204*. https://doi.org/10.1016/j.bcp.2022.115210

Reid, C. E., Brauer, M., Johnston, F. H., Jerrett, M., Balmes, J. R., & Elliott, C. T. (2016). Critical review of health impacts of wildfire smoke exposure. *Environmental Health Perspectives*, *124*(9), 1334–1343. https://doi.org/10.1289/ehp.1409277

Reynolds, S. J., Milton, D. K., Heederik, D., Thorne, P. S., Donham, K. J., Croteau, E. A., Kelly, K. M., Douwes, J., Lewis, D., Whitmer, M., Connaughton, I., Koch, S., Malmberg, P., Larsson, B.-M., Deddens, J., Saraf, A., & Larsson, L. (2005). Interlaboratory evaluation of endotoxin analyses in agricultural dusts—Comparison of LAL assay and mass spectrometry. *Journal of Environmental Monitoring*, *7*(12), 1371–1377. https://doi.org/10.1039/B509256F

Schaeffer, J. W., Reynolds, S., Magzamen, S., VanDyke, A., Gottel, N. R., Gilbert, J. A., Owens, S. M., Hampton-Marcell, J. T., & Volckens, J. (2017). Size, Composition, and Source Profiles of Inhalable Bioaerosols from Colorado Dairies. *Environmental Science & Technology*, *51*(11), 6430–6440. https://doi.org/10.1021/acs.est.7b00882

Shukla, S. D., Shastri, M. D., Jha, N. K., Gupta, G., Chellappan, D. K., Bagade, T., & Dua, K. (2021). Female gender as a risk factor for developing COPD. *EXCLI Journal*, *20*, 1290–1293. https://doi.org/10.17179/excli2021-4118

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APPENDIX A

List of Acronyms:

- AA – Arachidonic Acid
- ALOX-5 – Arachidonate 5-Lipoxygenase
- BBB – Blood-Brain Barrier
- CO_2 – Carbon Dioxide
- COX-2 – Cyclooxygenase-2
- COPD – Chronic Obstructive Pulmonary Disease
- ELISA – Enzyme-Linked Immunosorbent Assay
- FBS – Fetal Bovine Serum
- HBECs – Human Bronchial Epithelial Cells
- LTE_4 – Leukotriene E4
- LTs – Leukotrienes
- PBS – Phosphate Buffered Saline
- PGs – Prostaglandins
- PGE_2 – Prostaglandin E2
- PM – Particulate Matter
- PM_{10} – Particulate Matter ≤10 μm in aerodynamic diameter
- $PM_{2.5}$ – Particulate Matter ≤2.5 μm in aerodynamic diameter
- rtPCR/qPCR – Real-Time Polymerase Chain Reaction / Quantitative Polymerase Chain Reaction
- THP-1 – Human Monocytic Cell Line
- UFPM – Ultra fine Particulate Matter