DISSERTATION

# ASSESSMENT AND INTERVENTION STRATEGIES FOR AGRICULTURAL INHALATION EXPOSURES IN OCCUPATIONAL AND COMMUNITY ENVIRONMENTS

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# ABSTRACT

## ASSESSMENT AND INTERVENTION STRATEGIES FOR AGRICULTURAL INHALATION EXPOSURES IN OCCUPATIONAL AND COMMUNITY ENVIRONMENTS

Agriculture represents an industry vital to the U.S. economy, supplying the public with nutritious food and providing millions of workers with employment. Also characterized as one of the most hazardous industries for workers, agricultural environments contain a variety of inhalation hazards capable of impacting the health of workers and adjacent community residents. Agricultural inhalation hazards include airborne organic and inorganic dusts; livestock associated gases, pesticides, bacteria, viruses, and antibiotics. This study will focus on (1) bioaerosol exposures in dairy operations and (2) inorganic dust pesticide exposures from agricultural applications.

In dairy environments, workers are regularly exposed to high levels of organic dust (bioaerosols) and their inflammatory constituents (e.g., endotoxin, muramic acid, and β-glucans). Dairy bioaerosol exposure is associated with increased prevalence of respiratory disease (e.g., asthma, rhinitis, and chronic obstructive pulmonary disease) in dairy workers. While bioaerosol exposure in dairy environments has been well characterized in previous research, efforts to identify hygienic interventions that control exposure remain unsuccessful.

In crop production agriculture, it is well documented that workers are exposed to high levels of pesticides associated with adverse health outcomes (e.g., respiratory and neurologic diseases). Further, in agricultural adjacent community environments, where lower chronic pesticide exposures are found, there is mounting evidence linking adverse health effects (e.g., adult and

childhood cancers, neurologic and respiratory diseases, and birth outcomes) in residents to exposure from agricultural pesticide applications. However, weak and sometimes inconsistent associations previously reported highlight the limitations of current community exposure assessment techniques used for pesticides.

For specific Aim 1, we pilot tested a novel low-cost nasal rinse intervention to modulate airway inflammation in ten bioaerosol exposed dairy workers. Dairy workers were randomly split into treatment (n = 5) and control (n = 5) groups and administered saline nasal rinses before and after their shift for five consecutive days. Treatment group participants received pre-shift hypertonic saline rinses while the control group received normotonic saline rinses. Both received normotonic rinses post-shift. Pro and anti-inflammatory cytokines were measured from recovered saline rinses. Linear mixed model results indicated treatment group participants experienced significantly higher concentrations of anti- (IL-10) and pro-inflammatory cytokines (IL-6 and IL-8) than the control group ($p < 0.02$, $p < 0.04$, and $p < 0.01$ respectively). This study demonstrates the capacity of hypertonic saline nasal rinses to successfully upregulate anti-inflammatory cytokine production. However, conflicting upregulation of pro-inflammatory markers cloud interpretations of efficacy.

For Aim 2, we further evaluated the immunomodulatory effects of hypertonic saline rinses vs. normotonic saline rinses longitudinally (2-5 shifts) in 45 bioaerosol exposed dairy workers. However, in this aim, treatment group participants received hypertonic rinses pre- and post-shift and 16S sequencing was added to analyses to capture potential washout effects on microbial diversity. No significant differences were observed between group or day for any of the measured markers or microbiome diversity metrics. Yet, non-significant increases in anti-inflammatory IL-10 concentrations across the study period were observed independent of

treatment group suggesting the rinse itself may be more impactful than tonicity. This study provided mixed but encouraging results that justify further research on nasal rinses as an intervention in bioaerosol exposed dairy workers.

For Aim 3, we evaluated the agreement between three exposure assessment techniques used to estimate residential organophosphate (OP) exposure in agricultural adjacent communities located in the Central Valley of California. OP exposure was estimated from household dust samples, California Pesticide Use Report (CPUR) pesticide use modeling, and urinary DAP metabolites across two sampling campaigns. Simple correlation tests revealed moderate correlations ($\rho_s = 0.46$) between household dust and use model exposure estimates. Estimates from urinary DAP metabolites exhibited low to no correlation with the other two estimates. Linear mixed model results also indicated no association between urinary DAP metabolites and household dust or use model estimates. This study illustrates a lack of agreement between community pesticide exposure assessment techniques regularly used in research and motivates the development of more robust assessment techniques.

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# DEDICATION

Dedicated to my loving parents Mark and Kristi, amazing sister Hannah, and my incredible partner Francesca. Your support has meant the world to me.

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# CHAPTER 1: INTRODUCTION

The U.S agricultural industry employs millions of workers providing nutritious food and goods essential to everyday life [1,2]. With ever-increasing production demands, farms continue to increase in size to supply agricultural commodities and rely more and more heavily on a vulnerable migrant workforce [3]. Chemical, biologic, and physical hazards are regularly present in agricultural operations making it one of the most dangerous industries for workers [4,5]. Inhalation hazards have been identified as a particular concern by the National Institute for Occupational Safety and Health (NIOSH), as high prevalence of respiratory disease has consistently been observed in agricultural working populations [6–9]. Moreover, increased rates of adverse health effects from agricultural inhalation exposure have also been documented in agriculture adjacent communities [10–13]. In these environments a variety of organic and inorganic inhalation hazards are found including particulate matter, pesticides, bacteria, pathogens, antibiotics among others. The levels and contributions of each hazard is unique to the livestock and crops present in a given operation. Therefore, the research presented here specifically addresses bioaerosol exposures in dairy environments and organophosphate pesticide exposures in crop production environments.

In dairy operations, workers are subject to high levels of bioaerosols inherent to livestock operations [7,14,15]. Spanning the inhalable fraction (0-100μm), dairy bioaerosols exist as a mixture of particulate matter, allergens, microbial communities, and inflammatory constituents (e.g., endotoxin, muramic acid, and β-glucans) [15,16]. Inhaled bioaerosols impact in the upper airways and elicit pro-inflammatory responses. Further, in dairy environments, exposure is associated with increased risk of occupational asthma, allergic rhinitis, chronic obstructive pulmonary

disease (COPD), and respiratory pneumonitis [9,17–19]. This disproportionate burden of respiratory disease illustrates the need for hygienic interventions that mitigate bioaerosol exposure and respiratory inflammation.

Little research has been published on the implementation of interventions to control bioaerosol exposure in dairy operations, and prior efforts have been largely ineffective [20,21]. Interventional studies that did report encouraging results were considered too costly for dairy owners to implement [20,22]. In Aims 1 and 2 of this study we sought to address the limitations of previous interventions by investigating the efficacy of a novel, low-cost saline nasal rinse, to modulate the inflammatory response in bioaerosol exposed dairy workers.

In crop production, workers are disproportionally exposed to high concentrations of synthetic agricultural pesticides associated with increased risk of cancer, neurodegenerative conditions, and respiratory disease [23–26]. Off-target pesticide exposure from agricultural spraying has also been found to disproportionately impact residents in agricultural adjacent communities [27–29]. Further, community pesticide exposure from agricultural applications has been linked to a suite of health effects including adverse birth outcomes [30,31], adult and childhood cancers [13,32,33], neurodegenerative diseases [34,35], and respiratory disease [36,37]. Conversely, similar studies have reported no association between residential exposures and health outcomes [38–40]. Variable exposure assessment techniques used between studies may explain inconsistent associations reported in the literature via introduction of exposure misclassification [28,41]. Therefore, it is crucial to understand the relationships between different assessment techniques to inform robust future assessments. To address this, in Aim 3. we evaluated three exposure measurement techniques (household dust measurement, a group of six urinary metabolites, and GIS-based calculations) in communities adjacent to agricultural land.

The primary goals of this study were to explore an intervention in a well characterized agricultural environment (Aims 1 & 2) and evaluate exposure assessment techniques in a less defined community environment (Aim 3).

For Aim 1, we pilot tested the efficacy of a novel hypertonic saline nasal rinse in reducing nasopharyngeal inflammation in bioaerosol exposed dairy workers. Workers were administered either a hypertonic saline (treatment) or normotonic saline (control) nasal rinse before and after their shift across a five-day work week. Efficacy was assessed by comparing nasopharyngeal pro and anti-inflammatory cytokines between treatment and control groups over five days.

For Aim 2, we leveraged encouraging results from Aim 1 to conduct a more robust evaluation of hypertonic nasal rinses as an intervention in bioaerosol exposed dairy workers. Methodologically similar to Aim 1, nasopharyngeal inflammatory cytokines were measured from workers and compared between those receiving pre and post-shift hypertonic rinses and those receiving normotonic rinses across five days. A much larger sample size and expanded network of dairy locations distinguish Aim 2 from Aim 1.

For Aim 3, OP pesticide exposure was measured in agricultural adjacent communities in the Central Valley of California with three separate exposure assessment techniques. Exposure assessment techniques consisted of a pesticide use based exposure calculation, household dust collection, and urinary metabolites. Using correlations and linear mixed modeling, we examined relationships and the level of agreement between techniques to inform future research evaluating the effects of pesticide exposure in community environments.

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# CHAPTER 2: LITERATURE REVIEW

## United States Agriculture and Agricultural Workforce

The agricultural industry plays an integral role in providing accessible, nutritious food to the public. Agriculture is also a significant contributor to a nation's economy and supports the livelihoods of billions of people across the globe [1,2]. To meet increasing agricultural production demands, approximately 5 million workers are employed in the U.S. alone [3]. However, this essential industry has also been observed to be one of the most hazardous for employees. According to the U.S. Bureau of Labor Statistics, the rate of injury and illness cases was 4.1 per 100 full-time workers for agriculture, forestry, fishing, and hunting in 2022 [4]. The rate of injury and illness for all industries was significantly lower with 2.8 per 100 full-time workers [5]. In response, the National Institute for Occupational Safety and Health has made agriculture a significant component of their National Occupational Research Agenda.

The National Occupational Research Agenda (NORA) for Agriculture, Forestry, and Fishing was developed to help identify the research and information needed to prevent injury and illness in agriculture, forestry, and fishing workforces [6]. The agenda includes three objectives specific to agricultural workers: (1) reduce risk of fatal and nonfatal injuries to worker in the agricultural sub-sector; (2) reduce the risk of work-related illnesses to workers in the agriculture sub-sector; and (3) improve reporting and surveillance of injury and illness in the agricultural sub-sector, and reduce the risk of illness and injuries in vulnerable worker populations in the agriculture sub-sector [6]. This final objective has major implications for the agricultural industry in the U.S. that has historically relied on vulnerable populations like recent immigrants and foreign-born workers.

The agricultural workforce in the United States is largely comprised of Latino foreign-born workers. In the most recent National Agricultural Workers Survey (2019-2020) 78% of respondents were Hispanic with nearly two-thirds (63%) born in Mexico [7]. Additionally, nearly 50% of survey respondents did not have legal authorization to work in the U.S. while 52% didn't have health insurance [7]. Average education attainment among agricultural workers was one year of high school; mean and median income ranged from $20,000 to $25,000 [7]. These statistics provide a clear illustration of an at-risk migrant working population who have little formal education, low socioeconomic status, and lack access to healthcare. The demographic factors listed above can act synergistically with occupational hazards present in agricultural operations to exacerbate exposure risks and adverse health outcomes in workers. For instance, fear of deportation has resulted in a workplace environment where workers are afraid to report hazards and work-related injury and illness [8]. Fear of deportation, limited access to healthcare, and low-income work together to cultivate a workplace environment where injured and ill workers avoid seeking treatment [8,9]. Therefore, it is paramount that we understand the environmental threats present in agricultural operations and limit their impact on an at-risk working population.

In addition to worker demographics, over the past two decades, agriculture has shifted towards larger operations and has resulted in impacts on occupational exposure and health outcomes. U.S. agricultural production in the 21$^{st}$ century has transitioned from many smaller farms to fewer large scale specialized farms. According to the USDA Economic Research Service, the number of farms in the U.S. has declined from 2.2 million in 2007 to 2 million in 2022 [10]. During that same period the size of farms has continued to increase, peaking in 2022 at 446 acres [10]. Among dairy farms, consolidation to larger operations has been much more obvious as the median herd size has grown from just 80 in 1987 to 1300 in 2007 [11]. These dramatic shifts

towards larger scale farm operations require more hired workers leading to a greater dependence on at-risk migrant populations.

## Agricultural Inhalation Exposures

While dermal exposures and ingestion exposures are present in agriculture, inhalation exposures are of particular concern for worker health. The National Occupational Research Agenda specifically mentions respiratory hazards first, as part of its objective to "Reduce the risk of work-related illnesses to workers in the agriculture sub-sector" [6]. Farmworkers are exposed to a wide array of inhalation hazards including airborne organic and inorganic dusts, gases from livestock operations, pesticides, bacteria, and antibiotics [12,13]. These exposures are not homogenous across all agricultural operations. Instead, inhalation exposures can vary greatly depending between crop and livestock agricultural operations as well as within specific crops and livestock. Therefore, this paper will be concentrated on (1) organic dust exposure in dairy operations and (2) inorganic dust pesticide exposures in crop growing operations.

### *Dairy Bioaerosols*

Airborne organic dust, also known as airborne biological particulate matter or bioaerosols, as they will be referred to for the remainder of this document, are regularly present in dairy environments [14–19]. Fundamentally, dairy bioaerosols contain a mixture of allergens, viruses, and diverse microbial communities of bacteria, archaea, and fungi [15,16,20–23]. Upon cell death, these microbial communities can release inflammation generating components and toxins. Gram-negative bacteria release lipopolysaccharides (LPS) or endotoxin; gram-positive bacteria release muramic acid and peptidoglycans (PGN), and fungi release β-D-glucans [15,16,21,22]. In dairy environments, bioaerosols exhibit a wide range of shapes and sizes spanning the thoracic and inhalable fractions. The inhalable fraction encompasses particles with an aerodynamic diameter

less than 100um [17]. These larger particles impact in the nasopharyngeal region of the respiratory system that includes the nose, mouth, and larynx [24]. The thoracic fraction has been characterized as a sub-fraction of inhalable particles with an aerodynamic diameter cut point of 10um [25,26]. Particles in this sub-fraction are capable of penetrating beyond the larynx into the lower airways [25,26]. Consequently, characterization of exposure to dairy bioaerosols must consider viable and nonviable constituents that exist across a diverse size distribution.

Occupational exposure to inhalable bioaerosol dust in dairy environments has been well characterized in previous studies with varying measurement of specific components [19]. In the past decade, personal bioaerosol exposure in dairy environments has been assessed across at least 14 studies of varying size and complexity [19]. Across these studies, total inhalable bioaerosol dust geometric mean concentrations measured over the last 10 years ranged from 0.22 to 9.8 mg/m$^3$ [16,19–22,27,28]. Further, endotoxin exposure geometric mean concentrations measured in these studies during the same period ranged from 1.86 EU/m$^3$ to 1368 EU/m$^3$ [16,19–22,27,28]. Personal exposure to β-glucan was characterized in two studies investigating dairy environments with concentrations of 1.5 ng/m$^3$ and 14.3 ng/m$^3$ [21,28]. Peptidoglycan exposure was not measured among studies conducted during the 10-year period. Presently, contextualizing measured bioaerosol exposures to clinically relevant health outcomes remains difficult as no occupational regulatory standards currently exist. Instead, researchers and committees have previously proposed recommended exposure limits. These recommendations are not enforceable, but act as a best estimate to limit health effects in exposed workers. A study investigating dose-response relationships in bioaerosol exposed swine production workers published a recommended exposure limit of 2.5 mg/m$^3$ for bioaerosol dust [29]. For endotoxin, the Dutch Expert Committee on Occupational Safety suggested an occupational exposure limit of 90 EU/m$^3$ [30]. This limit was

based on a no observed effect level for adverse respiratory health symptoms [30]. Considering both limits are contained within the observed ranges of exposure above, dairy workers are overexposed to bioaerosol dust and endotoxin. Further, Davidson et al. found that 89% of dairy workers sampled from 30 U.S. dairies were above the 90 EU/$m^3$ recommendation [16]. A lack of regulatory guidance regarding enforceable exposure limits has allowed workers to be overexposed and develop a host of negative respiratory health outcomes.

*Health Impacts*

Increased prevalence of fixed airway diseases, respiratory allergy, and decreased lung function have consistently been observed in the dairy worker population. Pre-shift to post-shift or "cross-shift" decreases in lung function have shown promise as a marker for respiratory inflammation and may be predictive of long-term lung function changes [31]. Longitudinal studies have found duration of employment, smoking status, and age to be linked to decreases in respiratory function test metrics including one second forced expiratory volume (FEV1), forced vital capacity (FVC), and the ratio between the two (FEV1/FVC) [19]. However, recent efforts to link dairy bioaerosol exposures to acute cross-shift changes have produced mixed results. A study comparing baseline and cross-shift lung function in workers from large herd dairies to a control group working in a vegetable processing plant found that dairy workers had lower baseline function and experienced a greater cross-shift decline [32]. Almost one-third of dairy workers experienced ≥3% cross-shift decrement in $FEV_1$ (30%) and FVC (29%), while only 15% and 13% of control workers experienced ≥3% decrement to $FEV_1$ and FVC respectively [32]. Conversely, two separate studies investigating dairy worker pulmonary function in Iowa, Minnesota, Wisconsin, South Dakota, and the U.S. High Plains region reported negligible cross-

shift changes [20,28]. With more research, pulmonary function testing can be a useful tool to measure respiratory outcomes before onset of downstream fixed airway diseases.

Fixed airway diseases like occupational asthma, chronic bronchitis, and chronic obstructive pulmonary disease (COPD) have historically been recognized as a significant health burden in the dairy workforce. A recent review of dairy worker occupational allergy and respiratory diseases summarized the current literature on dairy workers and fixed airway diseases. They found that across nearly all included studies, dairy work was associated with an increased risk for asthma, chronic bronchitis, COPD, and asthma-like respiratory symptoms [19].

Occupational asthma, also known as work-related asthma, is a chronic inflammatory disorder of the airways caused by sensitization to a workplace contaminant [33]. Considering the high exposures to allergens and other sensitizing agents that make up bioaerosols, dairy environments represent a hotbed for occupational asthma. A longitudinal study measuring asthma prevalence changes among Swedish dairy farmers across a 12-year period [34]. After 12 years asthma diagnoses jumped from 2% to 8.9% which was higher than the general population prevalence in the same region [34]. In a more recent cross-sectional study, Stoleski et al. evaluated the prevalence of asthma in 83 dairy farmers against 80 matched office workers and reported rates of 7.2% and 5% for farmers and office workers respectively [35]. Even when compared to different agricultural operations, it appears dairy workers experience greater risk for occupational asthma. Eastman et. al. found that dairy workers were 2.73 times more likely to develop asthma than employees working at a vegetable processing plant [36].

COPD refers to a group of conditions caused by damage and inflammation in the airways that result in airflow blockages in the lungs making breathing difficult [37]. Conditions contained within the COPD umbrella include chronic bronchitis and emphysema. In dairy environments,

regular exposure to inhalable organic dust and its inflammagenic components are thought to cause the chronic inflammation required to develop COPD [18,38,39]. Studies investigating COPD prevalence among workers have followed the diagnostic criteria defined by the Global Initiative for Chronic Obstructive Pulmonary Disease (GOLD) [40]. The criteria leverage spirometry results to diagnose COPD and categorize severity as mild, moderate, severe, and very severe [40]. In a French study of male dairy farmers, Marescaux et al. measured COPD in 12.0% of participants [41]. They also found that smoking, a known cause of COPD, was associated with a higher prevalence of COPD among dairy farmers [41]. Another study comparing farm workers exposed to animal operations to those without found exposure to animal operations to be associated with 1.51 (95% CI 1.21, 1.89) times greater odds of COPD. Finally, a recent study of Macedonian dairy workers measured the impact of employment/exposure duration on COPD prevalence [42]. In dairy workers with over 20 years of dairy work exposures the odds of developing COPD were 36 times higher than workers with <20 years of exposure, OR = 36.00 (95% CI: 1.21-37.77) [42].

Evaluation of health effects linked to dairy bioaerosol exposure must consider the intrinsic factors that play a role in individual susceptibility. For instance, childhood exposure to bioaerosol dust has been shown to be protective against the onset of allergic sensitization and asthma. Danish farming school students who grew up on a farm between 1994 and 1998 had a reduced risk of developing adult-onset asthma than students who didn't (OR 0.5, 95% CI: 0.3-0.98) [43]. Specific farming practices may also convey protective effects against allergy and asthma. Stein et al. found that differences in asthma and immune responses among Amish and Hutterite communities were attributable to differences in farming practices [44]. Genetic differences among workers may also determine dairy bioaerosol susceptibility. For instance, a mutation in toll-like receptor-4 (TLR4) proteins, the receptor implicated in the pro-inflammatory

response to endotoxin, may reduce inflammation from inhaled endotoxin [45]. However, studies involving this mutation have observed contradicting results that require further investigation [46–48]. Polymorphisms in the CD14 receptor may also play a role in host response to endotoxin exposure. In a study of Dutch farmers, CD14 polymorphisms were associated with an increased inflammatory response and worse lung function after endotoxin exposure [49].

*Agricultural Pesticides*

Pesticides are used ubiquitously in crop agriculture to protect crops from harmful pests like weeds, insects, rodents, and fungi. Global pesticide use has rapidly grown over recent decades, impacting human health near and far from application sites [50–52]. In early agriculture, farmers relied on naturally occurring insecticides including pyrethrins, sulfur, and copper [53]. With the advent of synthetic pesticides, naturally occurring pesticides were largely replaced outside of organic operations [54]. Synthetic pesticides generally exhibit higher toxicity to targeted and non-targeted organisms than naturally occurring alternatives [55]. Therefore, synthetic pesticides will be the primary focus of this document. Due to their wide range of targets and applications, synthetic pesticides are regularly grouped based on chemical characteristics or the type of pest they control [56]. Major chemical classes include organochlorines, pyrethrins and pyrethroids, neonicotinoids, organophosphates, and carbamates.

*Organochlorine Pesticides*

Organochlorines make up a group of pesticides that contain chlorinated compounds. They are highly lipophilic, exhibit low water solubility, and are extremely persistent in the environment [57]. In exposed humans, organochlorines act as endocrine disruptors, and many have been shown to be carcinogenic and neurotoxic [58,59]. Because of their long-term persistence and impacts to human health, EPA banned the use of most organochlorines in the U.S. during the

1970s and 1980s [60]. However, some organochlorines banned by the European Union like 1,3-Dichloropropene (telone) continue to be widely used in the US [61].

*Pyrethrin Pesticides*

Pyrethrins are naturally occurring insecticides made by incorporating pyrethrolone and resin extracted from pyrethrum daisies. Similar to mechanism of action in organochlorine insecticides, pyrethrins block sodium channels in nerve axons to cause neurotoxic effects in target organisms [62]. Pyrethrins are highly unstable in the presence of UV light reducing their applicability in outdoor farming operations [62,63]. This limitation motivated the addition of functional groups to create more stable and potent synthetic derivatives called pyrethroids [62,64]. Modern pyrethroids are frequently used indoors and outdoors and are often used as replacements for banned insecticides [65–67]. Nonetheless, pyrethroids cause endocrine disruption and have been associated with impaired neurodevelopment, reduced reproductive function, and various cancers [64].

*Carbamate Pesticides*

Carbamates represent a class of pesticide derived from carbamic acid used as insecticides, herbicides, and fungicides. Unlike persistent organochlorines, carbamates exhibit low bioaccumulation potential and a relatively short biological half-life [68]. However, they are still considered hazardous enough to human and environmental health to be included in the US EPA priority list [68]. Carbamates act as anticholinesterases by reversibly carbamylating acetylcholinesterase receptors inhibiting the cessation of neurotransmitter signals [69]. This inhibition results in uncontrolled neurotransmitter signaling followed by excessive stimulation in the nervous system [69]. Human exposure to carbamates is associated with neurotoxicity, reproductive toxicity, and carcinogenesis [70].

*Organophosphate Pesticides*

Organophosphate pesticides are generally ester, amide, or thiol derivatives of phosphonic, phosphinic, and thiophosphoric acids [71,72]. They are widely used across the world due to their broad-spectrum efficacy and for their advantages over alternatives [73]. Unlike persistent organochlorines, OPs can rapidly degrade in natural environments where light is present [74]. Organophosphates act as acetylcholinesterase inhibitors in target organisms with a mechanism of action resembling carbamates [73]. Instead of reversibly inhibiting acetylcholinesterase by carbamylating receptors, organophosphates irreversibly inhibit acetylcholinesterase by phosphorylating the receptor [73]. The detrimental effects of OP exposure on the nervous system were recognized quickly after its discovery indicated by its use as a nerve poison during World War II [75,76]. Modern OPs used as pesticides have been developed to be less potent chemicals [75,76]. Yet, exposure to modern OP pesticides is still linked to neurodegeneration, neurodevelopment, reproductive toxicity, and carcinogenesis [77–82].

Organophosphate poisoning from acute exposure has historically been a major health concern. In 2020 it was estimated that approximately 740,000 unintentional pesticide poisonings occur annually [83]. According to the National Poison Data System 2020 annual report, only 2079 poisonings were documented in the U.S. [84]. However, these data relied primarily on self-reports and medical provider reports, likely underestimating true prevalence, especially in immigrant farm workers with little access to health care. The progression of symptoms observed in acute organophosphate poisonings have been well documented. Following acute exposure, clinical manifestations of toxicity are broken into three phases including cholinergic crisis, intermediate syndrome, and delayed polyneuropathy [85]. In the initial cholinergic crisis, acetylcholine accumulates in multiple locations in the body to resulting in salivation, muscle weakness,

abdominal cramps, headache, confusion, and, in severe cases, convulsions and flaccid paralysis [77]. Following cholinergic crisis, intermediate syndrome manifests between 1-4 days after exposure in the absence of initial symptoms [78]. Hallmark symptoms of intermediate syndrome are weakness in respiratory and proximal limb muscles [78]. In the 7-21 days after exposure, organophosphate-induced polyneuropathy can cause symmetrical weakness in the hands and feet [86].

While acute effects from OP exposure are better understood, there is a growing body of research exploring the health impacts from chronic exposure. Chronic occupational exposure to organophosphates has been associated with an increased risk for various cancers, respiratory diseases, and neurological impacts.

Respiratory health effects from long-term exposure to organophosphate pesticides range from decrements in lung function to more serious respiratory diseases like asthma, and COPD. Farmers with depressed acetyl cholinesterase activity, a common marker of OP exposure, have been shown to have greater reductions in pulmonary function [87]. In a study of 19,704 male U.S. farm workers, high pesticide exposure events were associated with an almost two times greater risk of allergic (OR 1.98, 95% CI 1.30-2.99) and non-allergic asthma (OR 1.96, 95% CI 1.49-2.56) [88]. Specifically, OPs parathion and coumaphos were associated with a 2.05- and 2.34-times greater risk for allergic asthma [88]. Further, a study of Canadian farmers found OP use associated with increased asthma prevalence [89]. The link between OP exposure and occupational COPD is less defined in the literature. Multiple studies have documented associations between occupational pesticide use and chronic bronchitis [90–92]. Researchers using data from the Agricultural Health Study from 2005-2010 found pesticide exposure to be associated chronic

bronchitis in farmers [91]. However, OP insecticide use was not consistently linked to COPD diagnosis [91].

Due to their inhibitory CNS function, chronic exposures to OPs have been implicated in the development of many neurological impairments. Neurobehavioral decrements to short term memory and attention have been observed in organophosphate exposed farmers [79,93–96]. Limited research has also observed a positive association between chronic OP exposure and Alzheimer's disease [97–99]. An association between OP exposure and Parkinson's disease risk has been suggested in a study of British sheep farmers [100]. Yet, the relationship was not significant (OR 1.56 95% CI 0.95-2.58) and acute poisoning was found to be a better indicator for development of Parkinson's [100].

Previous studies have indicated that farmers experience lower rates of lung, esophagus, bladder, liver, colon, and overall cancer [101–103]. Lower rates of smoking and higher physical activity levels found in farmers compared to the general population likely explain differences in cancer incidence [104]. Conversely, farmers have elevated rates of other cancers [101,104,105].

More than 100 studies worldwide have investigated the relationship between occupational pesticide exposure and prostate cancer risk [81]. In a meta-analysis of 15 studies conducted in North America, 8 found farmers were at increased risk for prostate cancer compared to non-farmers [106]. Although the other seven studies reported no association, subsequent publications from the Agricultural Health Study (AHS) have bolstered the proposed link. Koutros et al. observed significant associations between the highest organophosphate exposure category (Q4) and risk of aggressive prostate cancer compared to non-exposed individuals for fonofos (RR 1.63), malathion (RR 1.43), and terbufos (RR 1.29) in farm workers [107]. A recent publication also found the OP dimethoate to be associated with prostate cancer [108].

The relationship between occupational organophosphates exposure and development of lung cancer isn't fully understood. Farmers, as a whole, smoke far less than non-farming populations and experience lower rates of lung cancer [109]. The strong connection between smoking and lung cancer likely masks the effects of pesticide exposure in agricultural workers if not properly controlled [81,110]. Pesticide specific analyses from the AHS found OP use including diazinon, chlorpyrifos, and parathion to be associated with lung cancer occurrence [109,111,112]. However, observed associations have not been consistent and require further evaluation.

Hematologic malignancies represent a group of blood related cancers including leukemia, lymphomas, and non-Hodgkin lymphoma. A recent systematic review reported that occupational exposure to organophosphates increases risk of multiple hematologic malignancies [113]. Among 17 studies meeting methodological quality requirements, 14 supported an association between OP exposure and hematologic malignancies [113]. Evidence of an association was found for leukemia, lymphomas, non-Hodgkin's lymphoma, and multiple myeloma [113]. These findings should be considered with caution due to the limited number of studies included.

Increased risk for other cancers including kidney cancer [114] and breast cancer [115] have also been associated with occupational OP use. However, limited and conflicting associations require further research to better understand potential relationships.

In summary, several studies have suggested exposures to OPs and bioaerosols in occupational settings are associated with adverse neurologic, respiratory, and carcinogenic health outcomes. Many identified associations have been non-specific to pesticides within the OP chemical class or bioaerosol components limiting interpretation. Regardless, there is strong evidence that bioaerosols and OPs contribute to various acute and chronic health effects

observed in farmers. It is imperative that robust interventions and controls are implemented to reduce harmful occupational exposures and protect farm workers.

**Exposure Interventions**

Interventions that limit and control occupational exposures are essential to protect worker health and safety. The National Institute for Occupational Safety and Health (NIOSH) groups controls into five hierarchal categories based on efficacy [116]. In order from most to least effective, controls are categorized as elimination, substitution, engineering, administrative, and personal protective equipment (PPE). Elimination controls remove harmful exposures from the workplace. Substitution replaces a workplace hazard with a less harmful alternative. Engineering controls prevent worker contact with the occupational hazard without removing the hazard entirely. Administrative controls lower duration and frequency of exposure through scheduling and workplace task adjustments. Finally, PPE is equipment worn to reduce exposure to a hazard when no other control is feasible. When selecting and implementing an intervention or control, it is crucial to consider its economic feasibility, especially in agriculture where low socioeconomic status is common [7].

Interventions and controls surrounding pesticide exposure have traditionally relied on substitution indicated by the evolution of pesticide use over the last 100 years. These substitutions have mostly been the result of legislative restrictions and chemical bans. Historically, organochlorines like DDT represented a major proportion of pesticides used in the U.S. but were later phased out for less persistent alternatives like organophosphates [117]. Now with increasing evidence of the adverse health effects from organophosphate exposure, another shift to safer alternatives seems likely. EPA recently announced accelerated action on four OP pesticides based on updated exposure assessments to reduce use and exposure [118]. However,

these substitutions and restrictions have required decades of supporting research to be implemented. Research on alternative interventions is needed in the interim to protect at risk workers. A recent systematic review exploring the effectiveness of interventions to reduce pesticide exposure found that educational/behavioral based interventions represent much of the existing research [119]. The review found no studies specifically evaluating engineering controls or technological intervention. Additionally, interventions involving farmers and their families were more successful than interventions on farmworkers and their families. This discrepancy is likely attributable to farmworker demographics (low income, immigrant, lower education, and language barriers) [120]. Respirator use has also been investigated to limit exposure to pesticides but relies on worker behavior to be effective. In a survey on respirator use among farm operators only 54.5% of workers using pesticides used respirators [121]. Another study found that many workers who were aware of the importance of respirator use still refused to wear them [122].

Efforts to control bioaerosol exposure in dairy settings have been sparse and consistently exhibit concerns regarding economic feasibility [19,123–125]. To our knowledge, no studies have been conducted that assess the effectiveness of elimination or substitution controls in reducing bioaerosol exposures in dairy environments. Engineering controls have also received little attention, with only one recent publication. Islam et al. evaluated the effectiveness of a photocatalytic air cleaning device in reducing bioaerosol exposure in an enclosed dairy [125]. Installation of the air cleaning device resulted in an 8.05% and 61.56% reduction in 5-10um aerosols and *Escherichia coli* respectively [125]. Only one enclosed dairy was included in the study, therefore, generalizability of results to other operations is unclear. The lone study evaluating administrative controls, tested the impact of doubling parlor cleanings on bioaerosol concentrations [123]. Increased parlor cleaning resulted in a significant decrease in respirable dust

exposure, but not in inhalable dust or endotoxin. Finally, a study on respirator use in agricultural settings found respirators may provide reduced dust exposure, but are inadequate for bioaerosol exposures [124]. None of the aforementioned studies evaluated the costs associated with each intervention.

Research evaluating specific interventions to reduce inhalation exposure in crop and dairy farming operations is lacking. More precisely, a paucity of research has targeted elimination, substitution, and engineering controls that represent the most effective hierarchical control strategies. Future research should focus on these higher-level interventions that don't rely on worker behavior and training. Researchers should also work closely with dairy and crop producers to ensure interventions are not only effective, but low cost and feasible across the industry.

**Take-Home and Community Exposures**

Inhalation exposures from agricultural operations have mostly been considered an occupational problem for farmers and farm workers. However, the shift away from small family farms to large-scale operations may increase the risk of exposure and adverse respiratory health in rural community members [126,127]. Residents who once had a much larger buffer between themselves and crop and animal operations, now find themselves living adjacent to larger facilities. Additionally, agricultural workers have been shown to take-home exposures on their skin, clothes, vehicles, and shoes leading to "para-occupational" exposures for other housemates [128]. For children, residential exposures to pesticides and bioaerosols are particularly concerning because of the amount of time on floors where harmful dust settles, increased inhalation rates, and hand-to-mouth activities [128–130].

There is a mounting body of evidence reporting on the exposures and adverse chronic health effects associated with proximity of residents to agricultural operations [131–137]. Lower lung function and higher risk of respiratory symptoms, pneumonia, and lower respiratory tract diseases like COPD have all been found in residents proximal to livestock operations [131–137]. Conversely, studies by Smit et al. and Post et al. have reported lower prevalence of asthma and lower asthma and COPD prescription rates among residents nearby farms [138,139]. Results from studies involving crop production have, similarly, been mixed. Residential exposure to agricultural pesticide use has been linked to adult and childhood cancers (e.g., breast cancer, leukemia, and brain tumors)[140–147], neurodegenerative diseases (e.g., Parkinson's and amyotrophic lateral sclerosis)[148–151], impaired neurodevelopment [152,153], birth outcomes (e.g., pre-term birth and neural tube defects)[82,154–156], and respiratory health (e.g., asthma, COPD, lung function) [157–159]. However, other similar studies have reported weak relationships or no relationship between pesticide exposure and breast cancer [160–163], childhood asthma [164], birth outcomes [165–168], bladder cancer [169], and pre-term birth [170]. The variable outcomes observed in studies on residential pesticide exposure may be attributed to inconsistent and non-standardized exposure assessment techniques.

*Community Exposure Assessment*

Existing exposure assessment techniques used to estimate pesticide exposure in occupational environments generally consist of active sampling of short sample durations. These validated methods are inadequate to capture low-level chronic exposures thought to be present in communities near agricultural operations. Without a standardized method, community-based studies have employed questionnaires, GIS based models, household dust sampling, and

biomonitoring of metabolites; each of which is likely to introduce misclassification bias to varying degrees.

Questionnaires and surveys attempt to qualitatively estimate exposure through questions regarding household and occupational pesticide use as well as nearby spray events and proximity to agricultural operations. This technique relies on participants ability to recall the information being asked in the survey, introducing recall bias that can result in misclassification [171].

Assessment of pesticide exposure with GIS based exposure models has become a popular technique as it doesn't require extensive sampling campaigns in remote agricultural communities. Instead, models leverage pesticide application data and land use to estimate exposure in agriculture adjacent communities. GIS based assessment techniques are helpful to understand the potential household level pesticide exposure attributable to agricultural use but are very limited in estimating personal exposure. Without a complete understanding of household and personal factors that influence the amount of pesticide infiltrating the indoor environment, these estimates are less representative of indoor exposure. Additionally, the California pesticide use report used by most GIS based exposure assessments, operates on a multi-year delay. This limits its use to studies using previously collected data.

Household dust sampling represents another commonly used pesticide exposure assessment technique. Consisting of vacuuming up carpet dust with a specialized vacuum and analyzing it for pesticides, household dust can be used to assess indoor pesticide exposure. Carpet dust is particularly helpful for estimating exposure in children who are more likely to play on the ground and exhibit hand in mouth behaviors [128–130]. Yet, household dust can only be used to estimate pesticides bound to particulate matter (particle phase pesticides). Without a separate measure of vapor phase pesticides, exposure assessments using household dust only provide a

piece of the puzzle. Furthermore, household dust cannot provide any source apportionment information as it is the accumulation of pesticides from all sources.

Urinary metabolites are also frequently used to estimate pesticide exposure in community environments. Assessing exposure through metabolites can provide an accurate estimate of the internal dose residents receive. Even still, urinary metabolites are limited by a severe lack of specificity. Since they represent the total internal exposure to pesticides, they cannot reveal any information regarding the source of exposure. Frequently used metabolites like dialkylphosphates (DAPs) are also found in the environment meaning measured urinary concentrations cannot be exclusively attributed to OP exposure. Relating urinary metabolites to exposure from a specific pesticide is further complicated as many metabolites, including DAPs, are nonspecific to a single parent compound.

In summary, agriculture in the United States represents an ever-growing industry employing a substantial workforce characterized as a low-income, low-education, undocumented, and vulnerable population. This at-risk workforce is subject to countless occupational hazards including dairy bioaerosols and organophosphate pesticides. Occupational and residential exposure to agricultural bioaerosols and OP pesticides are associated with adverse cancer outcomes, respiratory disease, and neurologic outcomes. Important knowledge gaps exist for both of these exposures. Namely, effective interventions for well-characterized dairy bioaerosol exposures and exposure assessment techniques for OP exposure in agriculture adjacent communities. The work presented here adds to the literature, informs gaps in research, and provides recommendations for future investigations of agricultural inhalation exposures.

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# CHAPTER 3: PRELIMINARY INVESTIGATION OF A HYPERTONIC SALINE NASAL RINSE AS A HYGIENIC INTERVENTION IN DAIRY WORKERS


## Summary

Livestock workers experience an increased burden of bioaerosol-induced respiratory disease including high prevalence of rhinosinusitis. Dairy operations generate bioaerosols spanning the inhalable size fraction (0-100 μm) containing bacterial constituents such as endotoxin. Particles with an aerodynamic diameter between 10-100 μm are known to deposit in the nasopharyngeal region and likely affect the upper respiratory tract. We evaluated the effectiveness of a hypertonic saline nasal lavage in reducing inflammatory responses in dairy workers from a high-volume dairy operation. Inhalable personal breathing zone samples and pre-/post-shift nasal lavage samples from each participant over five consecutive days were collected. The treatment group (n=5) received hypertonic saline while the control group (n=5) received normotonic saline. Personal breathing zone samples were analyzed for particulate concentrations and endotoxin using gravimetric and enzymatic methods, respectively. Pro- and anti-inflammatory cytokines (i.e., IL-8, IL-10, and TNF-α) were measured from nasal lavage samples using a multiplex assay. Inhalable dust concentrations ranged from 0.15 to 1.9 mg/m$^3$. Concentrations of both pro- and anti-inflammatory cytokines, specifically IL-6, IL-8, and IL-10, were significantly higher in the treatment group compared to the control group ($p < 0.02$, $p < 0.04$, and $p < 0.01$ respectively). Further analysis of IL-10 anti-inflammatory indicates a positive association between hypertonic saline administration and IL-10 production. This pilot study demonstrates that hypertonic saline nasal lavages were successful in upregulating anti-inflammatory cytokines to support larger interventional studies.

## Introduction

Dairy workers have a high risk for exposure to bioaerosols, which have been consistently associated with inflammatory airway diseases [1–7]. Bioaerosols are complex mixtures containing particulates (primarily 10-100 μm in aerodynamic diameter) with an abundance of bacterial communities and associated inflammagens (e.g., endotoxin and muramic acid) that can impact in the nasopharyngeal region [7–9]. Previous studies have reported bioaerosol exposure concentrations as a high as 7.38 mg/m$^3$ – an exposure that is more than three times higher than occupational guidelines recommended by Reynolds *et al*. over 25 years ago (2.4 mg/m$^3$) [10,11]. Further, concentrations of endotoxin, a cell wall component of gram negative bacteria well-recognized to elicit inflammatory responses, were found to be in excess of the recommended occupational exposure limits including the former Dutch standard (90 EU/m$^3$) in 89% of dairy workers across 30 high plains dairies [6,12]. Consequently, dairy workers experience a disproportionate burden of respiratory disease that includes asthma, chronic obstructive pulmonary disease, and decreased lung functionality [3,4,13,14]. Moreover, symptoms of upper airway disease are largely overlooked (e.g., rhinitis), as 60-70% of agricultural workers report upper respiratory disease symptoms [15]. These upper respiratory symptoms often serve as precursors to asthma [8,9]. Increased surveillance could be important in prevention or early detection of lower airway symptoms and disease.

The dairy industry is unique when compared to other agricultural sectors due to its 24-hour production schedule, contact with large animals, operation of heavy equipment, and long work shifts [16,1>]. Moreover, dairy operations in the U.S. have transitioned to larger herds (>500 head) and shifted toward immigrant labor [16–19]. These dramatic changes to production, work organization and tasks have altered bioaerosol exposure patterns among dairy workers [16,17]. For example, to meet increasing demand, workers now have more specialized tasks (e.g.: milking,

bedding, and birthing), which we hypothesize has led to greater potential of sustained exposure to bioaerosols. The dairy workforce in the U.S. is estimated to be >90% Hispanic/Latino with no prior experience on a farm; this population is considered immunologically naïve to these complex bioaerosols [16,18–20]. As such, these workers may experience a more pronounced physiological response than those that have adapted to the environment. Many workers also have limited access to or interaction with medical care providers, highlighting the need for effective interventions to aid in health and welfare vulnerabilities of this at-risk population.

Interventions that follow a hierarchical approach to reduce respiratory illness among dairy workers have shown promise, but feasibility and effectiveness remain largely untested [21,22]. While engineering controls may be the most efficacious in reducing exposures, these solutions may not be economically feasible or practical. For example, reduction in personal exposure to respirable dust was observed after doubling the frequency of parlor washing, endotoxin concentrations were not reduced [22]. Further, costs associated with the large increase in water use was deemed a substantial adoption barrier. Personal protective equipment (e.g., N95 respirator) is a low-cost control; however, compliance is difficult to maintain and protection against bioaerosols has been shown to be inadequate [21]. In summary, the adoption and effectiveness of interventions to reduce worker inhalation exposures on dairies so far has been limited.

To address the limitations of past interventions, we pilot tested the effectiveness of a low-cost, low-burden nasal rinse among dairy workers exposed to high concentrations of bioaerosols. A potential therapeutic role for hypertonic saline solutions (HTS; a solution with elevated salt concentrations greater than physiological conditions) has been proposed as various *in vitro* and *in vivo* studies demonstrate its effectiveness in reducing pro-inflammatory markers in the lung when compared to a normotonic saline solution (a solution with physiologically normal salt

concentrations) [23–28]. However, its hygienic influence in the nasal passageway is untested and its efficacy on bioaerosol-exposed dairy workers remains unknown. We hypothesized that workers receiving the HTS nasal rinse would experience greater attenuation of pro-inflammatory cytokines and upregulation of anti-inflammatory cytokines than workers receiving a normotonic control with cumulative effects over time to inform interventional studies in these vulnerable populations.

## Methods

### *Participant recruitment*

A representative modern dairy operation in the southwest United States was identified and recruited through the HICAHS Dairy Advisory board. Study participation was offered to all workers employed at the dairy facility and based on their interest and availability to participate during the sampling period. From a pool of 20 eligible dairy workers, ten study participants with variable shift start times agreed to participate and were randomly assigned to the intervention or control group. All study protocols were approved by Colorado State University's (CSU) Institutional Review Board and informed consent was obtained in person prior to all data collection. Workers job tasks included milking, birthing, animal transport, mixing feed, re-bedding, animal medical care, and hoof maintenance. Worker personal protective equipment was limited (e.g., pants, long sleeves, and boots). After completion of the informed consent, participants were also administered a medical history questionnaire in either English or Spanish to determine if they met exclusion criteria (e.g., no use of steroidal nasal sprays, immune-suppressant medication, auto-immune medications, chemotherapy medications, recent surgery, recent chest injury, and history of stroke or heart disease).

Screening questionnaires were based on the American Thoracic Society (ATS) standard questionnaire with modifications specific to dairy populations [10,29,30].

*Sample Collection*

*Inhalable Dust*

Full eight-hour work-shift personal breathing zone (PBZ) inhalable dust samples were collected over all five sampling days using the SKC Button sampler equipped with 25mm PVC filters with 5um pore size (SKC Inc., Eighty-Four, PA). Samplers were connected to SKC XR5000 personal sampling pumps (SKC Inc., Eighty-Four, PA) that were calibrated to a flow rate of 4 L/min using a primary standard (DryCal Defender 510 Mesa Labs, Butler, NJ). A difference of +/-5% between pre- and post-sample flow rates were considered acceptable. Filters were desiccated for 24 hr before and after sampling prior to gravimetric analysis, which was performed using a Mettler MT5 balance (Mettler-Toledo, Columbus, OH). Field blanks were averaged and subtracted from sample weights before calculating airborne concentrations. Filters were stored at -80°C for subsequent endotoxin analysis.

*Endotoxin*

Filters were extracted in pyrogene-free water with 0.05% Tween-20. Filter extracts were analyzed using the Recombinant Factor C (rFC) assay (Lonza, Basel, Switzerland) on a Biotek reader (Biotek Instruments FLX800TBIE, Winooski, VT) as previously reported [2]. Endotoxin was quantified based on standard curve and fluorescence and reported as endotoxin units per cubic meter of air ($EU/m^3$). Reagent blanks, filter blanks, and quality control spikes were used to ensure accuracy of results.

*Nasal Lavage and Pro-Inflammatory Cytokines*

Pre- and post-shift nasal lavages were administered to participants across all five days of sampling. Participants in the interventional treatment group received normotonic saline solution (308mOsm) pre-shift and hypertonic saline (HTS) (400mOsm) post-shift to assess the effect of HTS post-exposure and not interfere with pre-exposure flora. Participants in the control group received normotonic saline solution pre- and post-shift. Once collected, a protease inhibitor was added (1% v/v) to lavage samples before storage on ice until processing. Lavages were centrifuged, aliquoted into 1ml supernatants, and stored in a -80°C prior to cytokine measurement. Meso Scale Discovery (MSD) technology was used to measure 10 cytokines and chemokines, including interleukin (IL)- IL-1β , IL-2, IL-4, IL-6, IL-8, IL-12p70, IL-13, interferon gamma, and tumor necrosis factor (TNF)-α, as well as IL-10 (an anti-inflammatory cytokine). Samples were analyzed and quantified with the MSD V-PLEX assay and MSD QuickPlex SQ 120 (MESO Scale Diagnostics, Rockville, MD).

*Statistical Analysis*

An exclusion criterion was applied to each analyte to mitigate the impact of non-detects on data analysis. Analytes with greater than 70% of measurements above the LOD (i.e., detectable measurements) were included in downstream analysis, while analytes with less than 70% detectable measurements were excluded. Analytes meeting this criterion had all non-detect measurements assigned a value equal to the LOD divided by the square root of two.

Based on the log normal distribution of the data, the bioaerosol exposure measurements were log transformed for statistical analysis. Descriptive measures [i.e., geometric means (GM) and geometric standard deviations (GSD]) were calculated and compared to suggested occupational exposure limits [11,12,31].

A statistical model was then used to relate exposure measurements (i.e., inhalable dust & endotoxin) to the measured outcome (cytokines) and determine the effect of the lavage intervention.

For dust and endotoxin, a linear mixed effects model was used to compare exposures between HTS treatment and control groups (fixed effect for group) across the five days of the study (fixed effect for day), and where applicable (i.e., the cytokine measures) the time of day (i.e., pre-shift, post-shift; fixed effect). Two-way interaction effects were included in all models and for cytokine measures, three-way interaction between group, day, and time of day was included in the model. A random effect for participant was included to account for repeated measures of the participants over the five days of the study. Log (base 10) was used to transform each of the measures to overcome heteroskedasticity of the residuals. Specifically, the model for dust can be expressed as:

$$y_{ijk} = \mu + group_i + day_j + group * day_{ij} + p_k + \epsilon_{ijk} \quad (1)$$

where $y_{ijk}$ is the log exposure level of the $k^{\text{th}}$ participant in the $i^{\text{th}}$ group (treatment, control) on day $j$ ($j$=1,…,5). The random effect for participant $k$ is represented by $p_k$ which follows a normal distribution with mean 0 and variance $\sigma_p^2$. $\epsilon_{ijk}$ represents the random error term that follows a normal distribution with mean 0 and variance $\sigma_\epsilon^2$. The model for the cytokines is the same as in (1) but also includes the fixed effect terms $time_of_day_m$, $time_of_day * day_{jm}$, $time_of_day * group_{im}$, and $time_of_day * group * day_{ijm}$ where time of day has two levels of am and pm. The variance components covariance structure was used in the estimation of the random effects. While this is a simplistic structure, due to small sample size, adding more parameters was not feasible.

SAS PROC MIXED with Kenward-Rogers degrees of freedom adjustment was used to fit each of the models. Tukey's Honestly Significant Difference (HSD) was used to account for multiple comparisons. All tests were conducted using a significance level of 0.05 in SAS (Version 9.4, Cary, NC) and R (Version 3.5.1., R core team, Vienna, Austria).

**Results**

A total of 100 nasal lavages and 50 PBZ samples were collected from a total of 10 participants across a five-day sampling campaign before and after worker shifts. Geometric mean dust concentrations for treatment and control groups were 0.45 mg/$m^3$ and 0.41 mg/$m^3$, respectively. Geometric mean endotoxin concentrations for treatment and control groups were 2.12 EU/$m^3$ and 1.59 EU/$m^3$ respectively. Daily inhalable dust and endotoxin concentrations separated by treatment group are presented in Table 3.1.

**Table 3.1**. Geometric mean (geometric standard deviation) dust measures for HTS treatment (T) and control groups (C) on each of the five days of the study. Mean based on n=5 for each group.

| Original Scale | Day 1 | | Day 2 | | Day 3 | | Day 4 | | Day 5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | T | C | T | C | T | C | T | C | T | C |
| mg/$m^3$ | 0.793 (1.457) | 0.394 (2.107) | 0.203 (6.258) | 0.318 (1.486) | 0.498 (2.339) | 0.531 (1.836) | 0.512 (2.150) | 0.524 (1.716) | 0.399 (1.927) | 0.317 (1.327) |
| EU/$m^3$ | 2.359 (4.687) | 3.406 (4.140) | 1.334 (8.098) | 0.945 (2.370) | 1.572 (3.628) | 0.994 (2.939) | 5.923 (3.144) | 2.102 (3.696) | 1.639 (2.299) | 1.528 (2.466) |

Among the ten chemokines and cytokines selected for analysis in the nasal lavage samples, five (TNF-α, IL-4, IL-12p70, and IL-13) were removed due to greater than 30% of values being below the limit of detection. Group level comparisons revealed the treatment group had significantly higher concentrations of IL-1β, IL-2, IL-6, and IL-8 pro-inflammatory cytokines (all $p<0.05$) as well as anti-inflammatory IL-10 ($p<0.01$). Log geometric mean concentrations by day, time, and treatment group are presented in picograms per milliliter

(pg/ml) in the figures below. Results from the linear mixed effects model fits, including tests of the fixed effects and estimates of the random effect are presented in Table 3.2.

There was no evidence of an interaction effect on average log mg/$m^3$ between treatment (HTS and control) and day (p=0.513), in average log mg/$m^3$ between HTS treatment and control groups (p=0.802), or among days (p=0.127), indicating similar exposures between groups. Additionally, there was no evidence of an interaction effect on average log EU/$m^3$ between treatment (hypertonic and control) and day (p=0.754), average log EU/$m^3$ between HTS treatment and control groups (p=0.634) or among days (p=0.114), also indicating similar exposures between groups.

There was evidence of an interaction effect on average log IL-10 between treatment, day, and time of day (p=0.009, Figure 3.1). After adjusting for multiple comparisons using Tukey's Honestly Significant Difference, there was evidence that the average IL-10 concentration in the treatment group was higher than the control groups after administration of HTS on two different days. Briefly, those concentrations observed on Day 3 (p=0.035/2=0.018; 95% confidence interval for average log IL-10 0.343, 1.10) and on Day 5 (p=0.002/2=0.001; 95% confidence interval for average log IL-10: 0.504, 1.26) indicate a positive association between HTS administration and IL-10 production.

**Table 3.2.** Linear mixed effects model fits for fixed effects and random effect estimates.

| | **Group (df=1)** | **Day (df=4)** | **Group*Day (df=4)** | **Time of Day (df=1)** | **Group* Time of Day (df=1)** | **Day * Time of Day (df=4)** | **Group*Day* Time of Day (df=4)** | $\widehat{\sigma}_p^2$ | $\widehat{\sigma}_\epsilon^2$ |
|---|---|---|---|---|---|---|---|---|---|
| Dust (mgm3) | F=0.07 (p=0.802) | F=1.95 (p=0.127) | F=0.84 (p=0.513) | | | | | 0.013 (0.02) | 0.100 (0.03) |
| Endotoxin (eum3) | F=0.25 (p=0.634) | F=2.04 (p=0.114) | F=0.47 (p=0.754) | | | | | 0.080 (0.07) | 0.228 (0.06) |
| Il10 | F=14.74 (p=0.005) | F=0.85 (p=0.496) | F=2.41 (p=0.057) | F=2.55 (p=0.115) | F=2.71 (p=0.104) | F=1.21 (p=0.313) | F=3.66 (p=0.009) | 0.007 (0.01) | 0.082 (0.01) |
| Il1b | F=0.88 (p=0.376) | F=0.84 (p=0.507) | F=1.89 (p=0.121) | F=18.19 (p<0.001) | F=0.74 (p=0.392) | F=0.75 (p=0.563) | F=1.61 (p=0.181) | 0.175 (0.10) | 0.174 (0.03) |
| Il6 | F=8.49 (p=0.020) | F=1.75 (p=0.149) | F=1.78 (p=0.141) | F=15.85 (p<0.001) | F=0.32 (p=0.574) | F=1.55 (p=0.197) | F=2.22 (p=0.075) | 0.063 (0.04) | 0.131 (0.02) |
| Il8 | F=5.97 (p=0.040) | F=0.24 (p=0.914) | F=0.94 (p=0.447) | F=1.41 (p=0.238) | F=0.26 (p=0.611) | F=0.67 (p=0.615) | F=1.49 (p=0.214) | 0.028 (0.03) | 0.367 (0.06) |

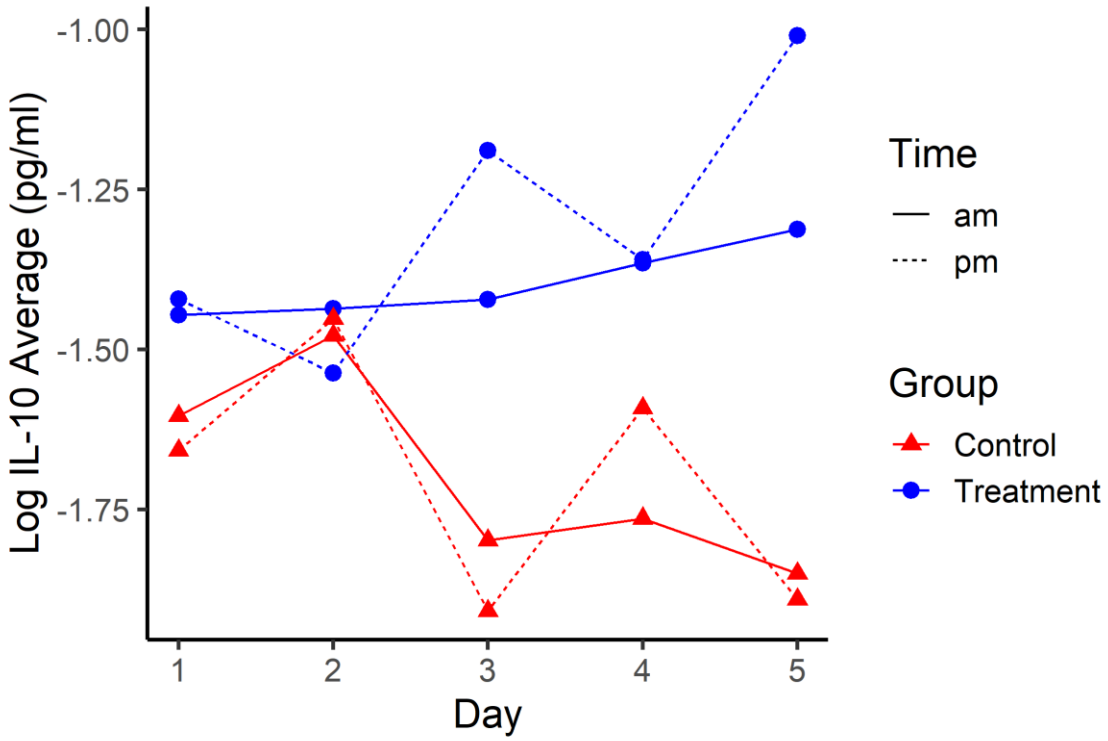



**Figure 3.1.** Average Log IL-10 across the five days for HTS Treatment (blue) and Normal Control groups (red) pre-shift (solid line) and post-shift (dotted line). Standard error for all estimates are 0.1339.

There was evidence of differences in average log IL-6 between treatment and control groups (p=0.020), with higher average concentrations being 0.106 to 0.912 units higher in treatment than control. There was also evidence of differences in average log IL-6 between pre- and post-shift measurements (p<0.001), with pm average log IL-6 being between 0.144 and 0.432 units higher than am average log IL-6 (Figure 3.2). There was not evidence of an interaction effect on average log IL-6 between treatment, day, and time of day (p=0.075), day and time (p=0.197), time and treatment (p=0.574), or day by treatment (p=0.141). There was not evidence of differences in average log IL-6 among days (p=0.150).

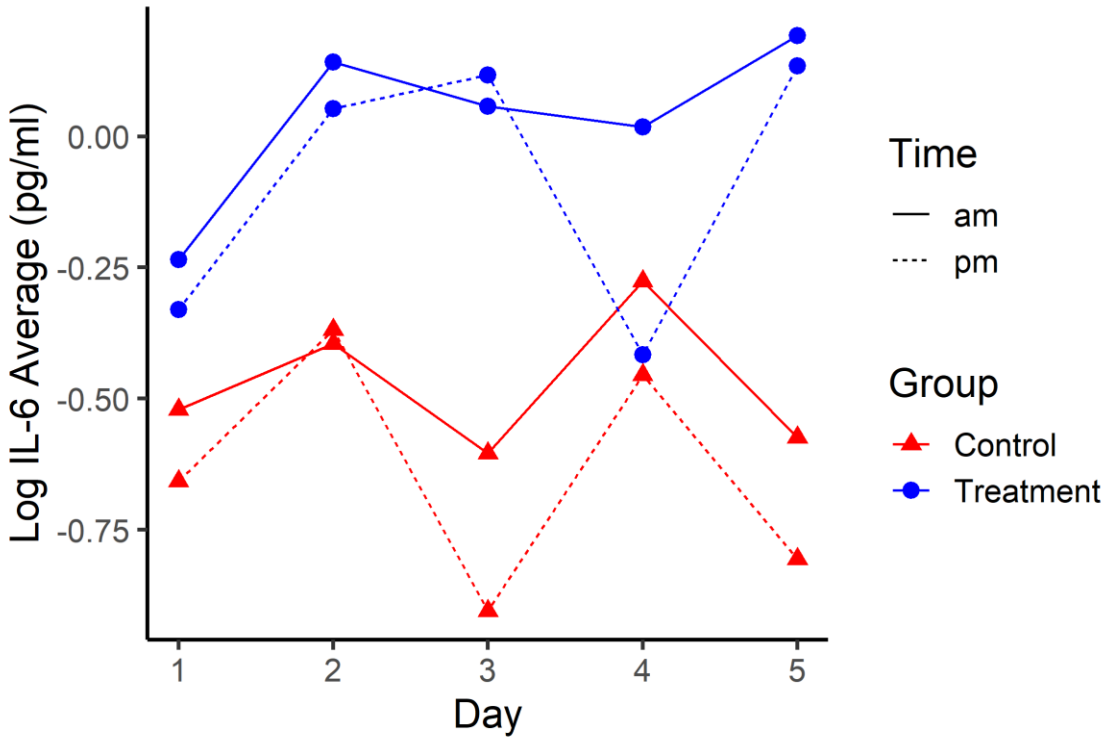



**Figure 3.2**. Average Log IL-6 across the five days for HTS Treatment (blue) and Normal Control groups (red) pre-shift (solid line) and post-shift (dotted line). Standard error for all estimates are 0.1236.

There was a difference in average log IL-8 between HTS treatment and control groups (p=0.040), with the estimated average log IL-8 for HTS treatment being between 0.022 and 0.763 higher than average log IL-8 for the control group (Figure 3.3). There was not evidence of an interaction effect on average log IL-8 between treatment, day, and time of day (p=0.214), day and time (p=0.615), time and treatment (p=0.611), or day by treatment (p=0.447). There was not evidence of differences in average log IL-8 among days (p=0.914) or between pre-shift and post-shift (p=0.238).

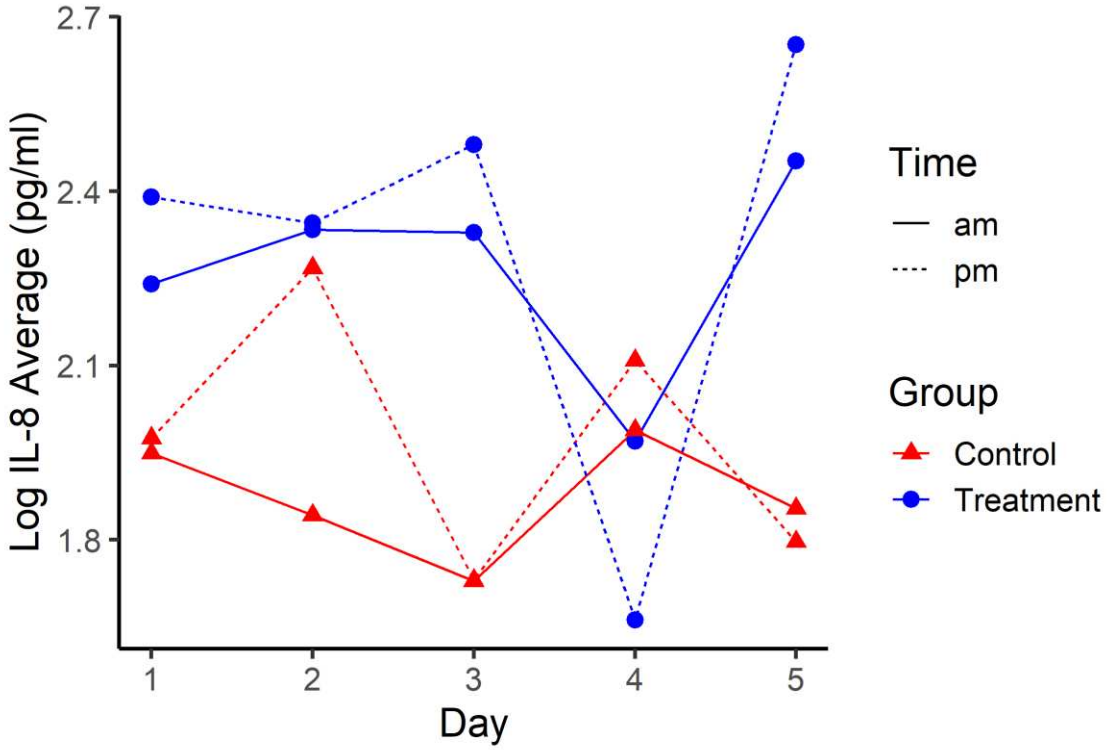



**Figure 3.3.** Average Log IL-8 across the five days for HTS Treatment (blue) and Normal Control groups (red) pre-shift (solid line) and post-shift (dotted line). Standard error for all estimates are 0.1135.

There was no evidence of an interaction effect on average log IL-1β between treatment, day, and time of day (p=0.181), day and time (p=0.563), time and treatment (p=0.392), or day by treatment (p=0.121). There was also not evidence of a difference in average log IL-1β between treatments (p=0.376) or among days (p=0.507). However, there was evidence of a difference in average log IL-1β between times of day (i.e., pre- and post-shift; p<0.001), with the pre-shift average IL-1β estimated to be between 0.190 and 0.523 units higher than the post-shift average log IL-1β (Figure 3.4).

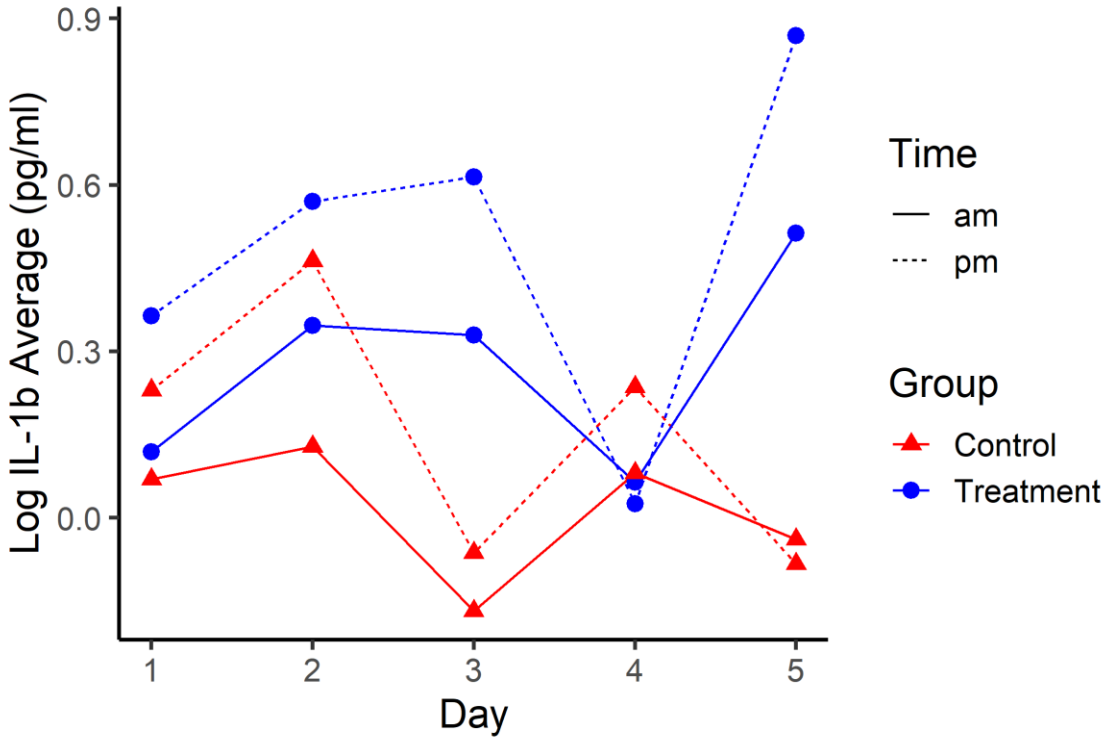



**Figure 3.4.** Average Log IL-1β across the five days for HTS Treatment (blue) and Normal Control groups (red) pre-shift (solid line) and post-shift (dotted line). Standard error for all estimates are 0.1448.

There were no differences in average log IL-2 between treatment and control groups, among days, or pre- and post-shift (all $p>0.05$). Therefore, IL-2 results are not presented here.

**Discussion**

Bioaerosols in large animal farming operations such as dairy facilities are associated with increase prevalence of both upper and lower airway diseases with limited therapeutic options. In this pilot study, hypertonic saline nasal lavages post-work exposure demonstrated potentially advantageous effects. The main interaction effects were observed among IL-10 concentrations, day, and time, supporting an increased anti-inflammatory effect with hypertonic saline irrigations as compared to normotonic saline lavages. However, there was no interaction effect for any of

the analyzed pro-inflammatory cytokines. Moreover, IL-6 and IL-8 pro-inflammatory cytokine levels were observed to be lower in controls than in the treatment group. It is important to note that higher baseline cytokine concentrations in the treatment group can be attributed to natural variation in immune response from participant to participant. For that reason, each participant served as their own control our analysis.

Our findings confirm previous clinical studies recently published on the immunomodulatory effects of hypertonic saline on anti-inflammatory cytokine production [24,25]. For example, adult hemorrhagic trauma patients treated with HTS plus dextran observed significant increases in production of anti-inflammatory cytokines IL-10 and IL-ra [25]. Others have demonstrated that peritoneal exudative macrophages preconditioned with HTS prior to lipopolysaccharide exposure exhibit a two-fold increase in IL-10 production as compared to isotonic pretreatment [24]. Collectively, our results and other studies strongly suggest that hypertonic saline treatment promotes upregulation of anti-inflammatory IL-10 release.

In contrast, HTS nasal lavage did not reduce commonly measured pro-inflammatory cytokine levels as has been reported in earlier studies. The aforementioned studies also observed decreases in IL-6 and TNF-α production with HTS while our results indicate an increase in IL-6 and partially in IL-8 [24,25]. IL-6 has been previously recognized as a pro-inflammatory mediator. Increased IL-6 levels in the serum and/or bronchoalveolar lavage fluid have been associated with asthma and COPD severity [32,33]. However, increases in IL-6 occur in active asthmatics even when TNF-α and IL-1β were not increased, which has led others to propose that IL-6 is not simply a pro-inflammatory marker but may be the result of "activated" airway epithelial cells [32]. Moreover, it has also been shown that airway inflammatory consequences induced by swine confinement facility organic dust extract exposure, which included airway neutrophil influx,

cytokine/chemokine release and lung pathology, were not reduced in IL-6 deficient mice as compared to wild type [34]. Thus, the significance of increased IL-6 following hypertonic saline solution administration necessitates further evaluation to fully understand its significance, particularly following large animal farming dust exposures. It is possible that a decrease in TNF-α existed in the treatment group, but too many samples were below the analytical limit of detection to adequately analyze.

In general, the concentrations of dust (0.46 mg/m$^3$) and endotoxin (1.94 EU/m$^3$) were overall lower than previously reported values and were also well below suggested exposure guidelines generated from studies of other agricultural environments [11,31]. These lower exposures could be caused by wet sampling conditions during the sampling period or cleaning measures used by this dairy facility. In either case, low exposure levels could be muting the effect size of HTS treatment, specifically in the pro-inflammatory cytokines directly associated with endotoxin exposure.

Alongside efficacy, it is also important to evaluate interventions through the context of adoption and use. A previous intervention introducing the use of N95 respirators was not well received by workers and inadequate compliance was noted (Lee et al. 2005). Because the nasal rinse can be completed within ~10 seconds before and after a shift, the barriers for adoption are much lower. The rinse will not interfere with job tasks, worker comfort, or require the worker to invest significant time before and after a work shift.

When interpreting the results from this study there are clear limitations to consider. First, sample size was small comparatively as only 10 dairy workers were enrolled. While repeated measures were utilized to achieve good sample depth, it is evident in the statistical analysis that the study did not have the power required to reveal small effects of additional inflammatory

mediator responses. Additionally, this study used a convenience sample by collecting exposure data from the first ten workers who volunteered. This could have led our study to be subject to healthy worker bias as workers who were on medication for underlying respiratory health problems were not eligible to participate due to testing requirements. We were also limited by the fact that this was a pilot study and the first to test the potential of a HTS nasal rinse as an intervention in the agricultural field. Nonetheless, these findings are an important proof-of-concept for optimizing sampling and analysis procedures. A lack of data on other immunologically reactive inflammagens (i.e., peptidoglycans) and health outcomes from the observed changes in pro- and anti-inflammatories is another limitation of this study. Future studies should characterize additional exposures and address the impacts of HTS on health-based outcomes like lung function and changes to the nasal microbiome. Finally, sampling from one dairy over a short time period in one season introduces generalizability limitations. Due to the lack of research on the application of HTS outside of laboratory and hospital settings, this study offers valuable insight for future studies despite these limitations.

**Conclusion**

Livestock workers experience an increased burden of bioaerosol exposure and subsequent respiratory disease with limited efficacious treatment options. The novel application of HTS nasal lavage in dairy workers following their work shift over a short period of time demonstrated advantageous results consistent with emerging data using HTS. Workers receiving the HTS rinse experienced significantly higher levels of the anti-inflammatory cytokine IL-10 than the control group. However, nasal levels of IL-6 and to a lesser degree IL-8 were increased in the treatment group. Consequently, there remains uncertainty regarding the effectiveness of HTS as a viable treatment option for respiratory disease in dairy workers. Based on these results, normotonic

saline rinses alone may provide health benefits and adequate attenuation against inflammation. Future studies are warranted with increased worker sample size as well as a pre- and post-shift HTS administration (as opposed to post-shift only) to improve statistical power and better quantify the practicality of the intervention. This study provides an important step towards utilizing low-cost, non-invasive approaches including this HTS nasal lavage strategy to improve the health of at-risk agriculture workers.

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# CHAPTER 4: HYPERTONIC SALINE NASAL RINSE INTERVENTION: IMMUNOMODULATORY EFFECTS IN DAIRY WORKERS


## Summary

*Objective*: Increased risk of occupational exposure to bioaerosols has long been recognized in livestock operations including dairy facilities. Spanning the inhalable fraction (0-100 μm), dairy bioaerosols are comprised of a wide variety of inflammatory components that deposit in the nasopharyngeal region. The resultant inflammatory response from bioaerosol exposure is likely driving the increased prevalence of respiratory disease observed in dairy workers. It is also thought that the microbiome of the upper respiratory system may help mediate this inflammation. We investigated the viability of a low-cost hypertonic saline nasal rinse intervention in modulating inflammatory responses in bioaerosol exposed dairy workers and its impact on microbial diversity.

*Methods*: Pre- and post-shift nasal rinses were administered and collected alongside full shift inhalable personal breathing zone (PBZ) samples for each participant up to five consecutive days. Treatment group participants (n=23) received hypertonic saline rinses while control group participants (n=22) received normotonic saline rinses. Particulate matter (PM) and endotoxin concentrations were quantified from PBZ samples using gravimetric and enzymatic analytical methods, respectively. Pre- and post-shift rinses were analyzed for pro- and anti-inflammatory markers and microbial diversity using a multiplex assay and 16S rRNA sequencing, respectively.

*Results:* PM and endotoxin concentrations were comparable between groups indicating similar exposures. Post-shift pro-inflammatory markers were significantly higher than pre-shift for IL-13 ($p=0.047$), IL-1β ($p<0.001$), IL-6 ($p<0.001$), IL-8 ($p<0.001$), and TNF-α ($p=0.024$).

There was no evidence of a difference in log concentrations between intervention group or day among any of the measured inflammatory markers. Anti-inflammatory IL-10 concentrations increased across the five sample days, independent of treatment group suggesting tonicity may not be driving the change. However, this result was not significant (p=0.217). Nasal microbiome alpha (within sample) and beta (between sample) diversity metrics did not differ significantly between group or day demonstrating no adverse washout intervention effects.

*Conclusion:* This study provided encouraging results that warrant future research to further evaluate saline nasal rinses as a workplace intervention.

## Introduction

Dairy workers are at an increased risk of exposure to airborne biological particulate matter (bioaerosols) commonly found in livestock operations [1–6]. Dairy-specific bioaerosols exist as complex mixtures of dust and biologically derived particles, including allergens (e.g., dander), microbial communities, and pro-inflammatory constituents (e.g., endotoxin, muramic acid, and β-glucans) [3,7–9]. Spanning an aerodynamic diameter of 10 μm to 100 μm, bioaerosols encompass the inhalable fraction that impacts in the nasopharyngeal region (e.g., nose, mouth, and pharynx) [4]. Once inhaled, these microbial constituents have the capacity to elicit inflammatory responses in the upper airways and are likely driving the increased prevalence of rhinosinusitis and contributing to chronic obstructive pulmonary disease (COPD), asthma, allergic rhinitis, and respiratory pneumonitis well-documented in dairy workers [10–14]. Furthermore, bioaerosol exposure seems to significantly shift nasal microbiome composition in workers' noses and may provide protection against pathogens [6,15–18].

Previous studies have demonstrated that bioaerosol exposure concentrations in dairy operations can vary by several orders of magnitude, ranging from 0.22 to 9.8 mg/m$^3$ [3,8,9,19,20].

Currently, no enforceable standards exist for occupational bioaerosol exposure or its inflammatory constituents, making the clinical relevance of measured exposures difficult to elucidate. However, a recommended exposure limit for bioaerosols of (2.4 mg/m$^3$) was suggested in a publication by Reynolds et al. [21]. Furthermore, the Dutch Expert Committee on Occupational Safety developed the former Dutch standard for endotoxin, an inflammatory component of gram-negative bacterial cell walls, of 90 EU/m$^3$ [22]. Previous research comparing worker endotoxin exposures across 30 High Plains dairies found that 89% of sampled workers exceeded the former Dutch standard [3]. Based on reported concentrations, dairy workers are exposed to bioaerosols and inflammagens exceeding recommended occupational limits resulting in a disproportionate burden of respiratory diseases and chronic allergies. Consequently, bioaerosol exposure and efforts to mitigate it continue to be a major industry concern.

Dairy industry practices including 24-hour production schedules, extended work shifts, contact with livestock, and use of heavy equipment create a unique exposure environment compared to other agricultural industries [23,24]. A notable shift towards larger operations working with bigger herds (>500 head) has altered production scale and dramatically changed the workplace dynamics of dairy operations [23–26]. Drastic shifts in worker demographics have also contributed to these challenges, as increased reliance on an under experienced, immunologically naïve immigrant workforce (>90% Hispanic/Latino) has led to a highly exposed and highly susceptible workforce [23,26,27].

Interventions attempting to control bioaerosols exposure and resultant respiratory illnesses in dairy settings have been encouraging, but major challenges regarding long term efficacy and economic feasibility remain [6,28,29]. Through guidance from the High Plains Intermountain Center for Agricultural Health and Safety (HICAHS) Advisory Board, we

identified a low-cost intervention with the potential to improve dairy worker respiratory outcomes. Previously, we pilot tested the effectiveness of a hypertonic saline (HTS) nasal lavage [19], based on previous *in vitro* and *in vivo* studies, to modulate the pro- and anti-inflammatory response [30–36]. Dairy workers receiving HTS nasal rinses across five consecutive days experienced a significant increase in anti-inflammatory cytokines compared to those receiving a normotonic saline (NTS), but also produced conflicting increases in two pro-inflammatory cytokines [19]. We aimed to build off of this result with a larger cohort to better understand the immunomodulatory effects of HTS in dairy workers.

Potential side effects from regular nasal rinses on the resident microbial communities present in the nose were not evaluated in the pilot study. Diverse resident microbiomes help maintain the stability and health of their environment, in this case the upper airways [37,38]. Previous studies have demonstrated a role for microbes and pathogens in the development of mucosal inflammation [39,40]. Therefore, perturbations to microbial diversity from hypertonic rinses could compromise stability and have adverse inflammatory effects. Consequently, the viability of HTS rinses as an intervention for respiratory disease in dairy workers required further testing in a larger cohort. We hypothesized that administering pre-and post-shift HTS nasal rinse to dairy workers would reduce the pro-inflammatory cytokine response and increase anti-inflammatory production when compared to an NTS control rinse. We also hypothesized the effect would be cumulative and would not cause perturbations or washout resident protective microbiota.

## Methods

*Participant recruitment*

We identified and recruited representative modern dairy operations across Colorado and Texas through the HICAHS Dairy Advisory board. Participation in the study was offered to all dairy facility employees with availability during sampling campaigns. The Colorado State University Institutional Review Board approved all study protocols and language specific informed consent was obtained in person before data collection (IRB approval #3015). A total of 45 study participants with varying shift times and work weeks agreed to participate and were randomly assigned to the intervention group (n = 23) or the control group (n = 22). Participant job tasks involved milking, birthing, insemination, office work, re-bedding, animal medical care, and animal transport. Personal protective equipment among workers was limited (e.g., long sleeves, boots, thick material pants, and sometimes cloth masks). Following informed consent, participants completed a medical history questionnaire containing exclusionary criteria in English or Spanish. Example exclusionary criteria included use of steroidal nasal sprays, immunosuppressant and autoimmune medications, recent surgery, chest injuries, and history of heart disease or stroke. Daily pre- and post-shift health and job task questionnaires were administered to record changes in symptoms among participants and animal contact time respectively. All questionnaires were based on the American Thoracic Society (ATS) standard questionnaire with dairy specific modifications.

*Sample Collection*

*Inhalable Dust*

Full work-shift personal breathing zone (PBZ) inhalable dust samples were collected from each participant for up to five consecutive sampling days using the SKC butting sampler

(SKC Inc., Eighty-Four, PA). Button samplers were equipped with 25 mm, 5 μm pore size PVC filters and connected to SKC XR5000 sampling pumps (SKC Inc., Eighty-Four, PA) calibrated to 4 L/min using a primary calibration standard (DryCal Defender 510 Mesa Labs, Butler, NJ). A pump flow rate drift less +/-5% between pre- and post-calibration was considered acceptable. Filters were dried in a desiccator 24hrs prior to gravimetric analysis before and after sampling. Gravimetry was performed with a Mettler MT5 balance (Mettler-Toledo, Columbus, OH). Field blanks were averaged and used to blank subtract sample weights before concentration calculations. Post-gravimetry, samples were stored in -80°C for downstream endotoxin analysis.

*Endotoxin*

Inhalable dust was extracted from PBZ filters in pyrogene-free LAL water with 0.05% Tween-20. Filter extracts were processed through the Recombinant Factor C (rFC) assay (Lonza, Basel, Switzerland) on a Biotek reader (Biotek Instruments FLX800TBIE, Winooski, VT) as previously reported [2]. Endotoxin quantification was based on level of fluorescence and reported in endotoxin units per cubic meter of air ($EU/m^3$). Reagent blanks, filter blanks, and quality control spikes were used to ensure accuracy of results.

*Nasal Lavage and Pro-Inflammatory Cytokines*

Participants were administered nasal lavages pre- and post-shift for up to five consecutive workdays. Intervention group participants received hypertonic saline solution (400mOsm) while control group participants received a normotonic saline solution (308mOsm). After collection, a protease inhibitor was added (1% v/v) to lavage samples before being put on ice and subsequent storage in a -80°C freezer. Lavages were vortexed and aliquoted into 1ml supernatants prior to cytokine and microbiome analysis.

Lavage aliquots were analyzed via Meso Scale Discovery technology (MSD) for 10 cytokines and chemokines, including interleukin (IL) -1β, IL-2, IL-4, IL-6, IL-8, IL-12p70, IL-13, interferon gamma (IFN-γ), tumor necrosis factor (TNF)-α, and the anti-inflammatory cytokine IL-10. Samples were analyzed and quantified with the MSD V-PLEX assay and MSD QuickPlex SQ 120 (MESO Scale Diagnostics, Rockville, MD).

Nasal lavage aliquots were analyzed using 16S rRNA sequencing technology to characterize the microbial composition of workers nasal microbiome at the University of Oklahoma Health Sciences Center. Samples were extracted and isolated using Zymo research Quick-DNA kits (Irvine, CA). Libraries were prepared with IDT DNA Technology primers and KAPA reagents according to the Illumina 16S Metagenomic Sequencing Library Prep protocol. PCR amplification of the 16S rRNA V3 and V4 regions was conducted with 5 ng of genomic DNA to construct individual libraries. Libraries were indexed for multiplex sequencing and sequenced with a MiSeq 600 Cycle Reagent Kit v2 on the Ilumina MiSeq platform. Resulting 300 bp paired end reads were processed in qiime2-2022.2 (q2)[41]. Sequence reads were denoised and organized into a feature table using the q2 DADA2 plugin[42]. Taxonomic classifications of amplicon sequence variants (ASV) were performed with a custom Naïve Bayes trained Silva classifier. Finally, a phylogenetic tree was created from the SILVA 128 SEPP reference database with the q2 sepp-fragment insertion plugin[43].

*Statistical Analysis*

To limit the effect of sample concentrations below the limit of detection on data analysis, an exclusion criterion was applied to each analyte. In cases where more than 70% of measurements were above the LOD (e.g., detectable concentrations), the analyte was evaluated statistically. Analytes with less than 70% of measurements above the LOD were excluded from

further analysis. Among analytes where more than 70% of measurements were above the LOD, all non-detect measurements were assigned a value equal to the LOD divided by the square root of two.

Bioaerosol exposure concentrations exhibited a log normal distribution and were log transformed for statistical analysis. Geometric means (GM) and geometric standard deviations (GSD) were calculated and compared to previously suggested occupational exposure limits [21,22,44]. A statistical analysis was conducted to determine the effect of the intervention on the measured inflammatory markers.

A linear mixed effects model was used for both dust and endotoxin to compare exposures between study variables to determine if exposures were consistent between treatment groups and sample days. Model fixed effects included group (treatment and control) and day (sampling day 1-5) with a random effect for participant to account for repeated measures. Two-way interaction effects were included in both models. All dust and endotoxin measurements were Log (base 10) transformed to address heteroskedasticity of residuals. Dust and endotoxin models are expressed as:

$$y_{ijk} = \mu + group_i + day_j + group * day_{ij} + p_k + \epsilon_{ijk} \quad (1)$$

where $y_{ijk}$ represents the log exposure level of the $k^{th}$ participant in the $i^{th}$ group (treatment, control) on day $j$ ($j$=1,…,5). Random effect for participant $k$ is denoted as $p_k$ which exhibits a normal distribution with mean 0 and variance $\sigma_p^2$. $\epsilon_{ijk}$ represents the random error term that follows a normal distribution with mean 0 and variance $\sigma_\epsilon^2$. Exposure model results were used to establish whether dust and endotoxin measures would be included as a variable in the outcome model below.

An outcome model was built for each cytokine and used the same fixed and random effects as in (1) but also includes the fixed effect term $time_of_day$ and subsequent interaction terms $time_of_day * day_{jm}$, $time_of_day * group_{im}$, and $time_of_day * group * day_{ijm}$ where time of day has two levels of am and pm. A variance component covariance structure was used in the estimation of the random effects.

Each of the mixed models were fit according to the Kenward Rogers method using the R package 'afex'[45]. Model estimated marginal means and associated confidence intervals were plotted to investigate and interpret interactions. All tests were conducted using a significance level of 0.05 in R (Version 4.3.0)[46].

*Metagenomics Analysis*

Statistical testing of nasal microbiome alpha and diversity metrics to determine differences in the intervention group and the control group was conducted with the core metrics pipeline in QIIME2 software using a significance level of 0.05. Sample alpha diversity was estimated with the q2 breakaway package[47]. Differences in alpha diversity between treatment group, day, and time were tested for with pairwise Kruskal-Wallis tests. Beta diversity estimates were determined with the q2 DEICODE package[48] and compared with pairwise PERMANOVA testing to determine significant differences between group, day, and time.

**Results**

A total of 45 dairy workers participated in the study for up to five consecutive days at five dairies in the High Plains region. With participation ranging from one to five days, 344 nasal lavages and 178 PBZ samples were collected and analyzed. Geometric mean dust and endotoxin concentrations for the treatment group were 0.36 mg/m$^3$ and 52.99 EU/m$^3$, respectively. Geometric mean dust and endotoxin concentrations for the control group were 0.36 mg/m$^3$ and

56.81 EU/$m^3$, respectively. PBZ dust and endotoxin concentrations stratified by day and treatment group are presented in Table 4.1.

**Table 4.1:** Geometric mean (geometric standard deviation) dust and endotoxin concentrations by treatment group and day.

| | Dust ($mg/m^3$) | | Endotoxin ($EU/m^3$) | |
|---|---|---|---|---|
| | Treatment | Control | Treatment | Control |
| Day 1 (N = 45) | 0.316 (3.35) | 0.369 (3.02) | 69.82 (6.28) | 39.27 (9.61) |
| Day 2 (N = 43) | 0.299 (3.11) | 0.336 (2.44) | 36.18 (9.33) | 51.29 (5.36) |
| Day 3 (N = 39) | 0.468 (4.61) | 0.324 (2.69) | 60.07 (9.43) | 54.55 (4.49) |
| Day 4 (N = 27) | 0.412 (3.84) | 0.356 (2.57) | 101.92 (4.72) | 84.18 (4.28) |
| Day 5 (N = 18) | 0.347 (5.27) | 0.472 (2.47) | 23.65 (8.64) | 75.83 (5.92) |

From the ten pro- and anti-inflammatory biomarkers measured from each nasal lavage, IFN-γ, IL-12p70, and IL-4 were removed for not meeting our criteria requiring at least 70% of values to be above the limit of detection. Group level comparisons among the remaining seven biomarkers indicated concentrations of TNF-α were significantly higher in the treatment group compared to the control group (p=0.018). Log adjusted biomarker concentrations averaged by treatment group, day, and time are presented in picograms per milliliter (pg/ml) in Figures 4.1-4.4 below. A summary of results from the linear mixed effects model fits are presented in Table 4.2.

**Table 4.2:** Linear Mixed Effect Model Results by Analyte

| | Group (df=1) | Day (df=4) | Time of Day (df = 1) | Group*Day (df=4) | Group*Time of Day (df=1) | Day*Time of Day (df=4) | Group*Day*Time of Day (df=4) |
|---|---|---|---|---|---|---|---|
| Dust | F=0.01 (p=0.950) | F=0.38 (p=0.826) | | F=0.54 (p=0.703) | | | |
| Endotoxin | F=0.08 (p=0.781) | F=0.62 (p=0.651) | | F=0.91 (p=.458) | | | |
| IL-10 | F=0.55 (p=0.463) | F=1.45 (p=0.217) | F=0.06 (p=0.807) | F=0.45 (p=0.774) | F=0.44 (p=0.510) | **F=4.01 (p=0.004)** | F=0.10 (p=0.982) |
| IL-13 | F=0.92 (p=0.344) | F=0.50 (p=0.739) | **F=4.00 (p=0.047)** | F=0.49 (p=0.745) | F=3.78 (p=0.053) | F=1.48 (p=0.209) | F=0.32 (p=0.866) |
| IL-1b | F=0.22 (p=0.644) | F=0.79 (p=0.534) | **F=0.71.22 (p=0.001)** | F=1.15 (p=0.332) | F=0.36 (p=0.550) | F=41 (p=0.803) | F=0.40 (p=0.807) |
| IL-2 | F=1.11 (p=0.298) | F=1.99 (p=0.096) | F=2.82 (p=0.094) | F=0.91 (p=0.457) | F=0.69 (p=0.407) | F=0.83 (p=0.507) | F=0.92 (p=0.451) |
| IL-6 | F=0.34 (p=0.565) | F=0.57 (p=0.682) | **F=14.36 (p=0.001)** | F=1.17 (p=0.326) | F=0.02 (p=0.896) | F=1.17 (p=0.326) | F=0.20 (p=0.940) |
| IL-8 | F=0.16 (p=0.691) | F=1.28 (p=0.279) | **F=14.29 (p=0.001)** | F=0.68 (p=0.610) | F=1.05 (p=0.307) | F=1.39 (p=0.236) | F=0.76 (p=0.550) |
| TNF-α | F=0.26 (p=0.612) | F=1.11 (p=0.352) | **F=5.16 (p=0.024)** | F=1.57 (p=0.183) | F=1.68 (p=0.197) | F=1.17 (p=0.323) | F=0.26 (p=0.902) |

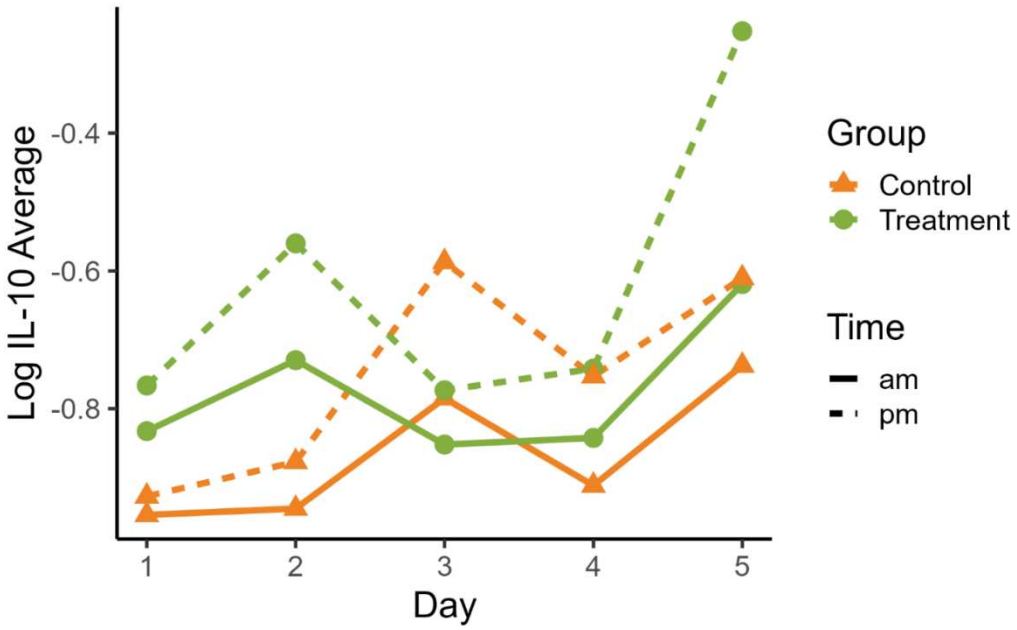



Figure 4.1: Average Log IL-10 across the five days for HTS Treatment (green) and NTS Control groups (orange) pre-shift (solid line) and post-shift (dotted line). Log IL-10 standard deviations ranged from 0.1035 to 0.6497.

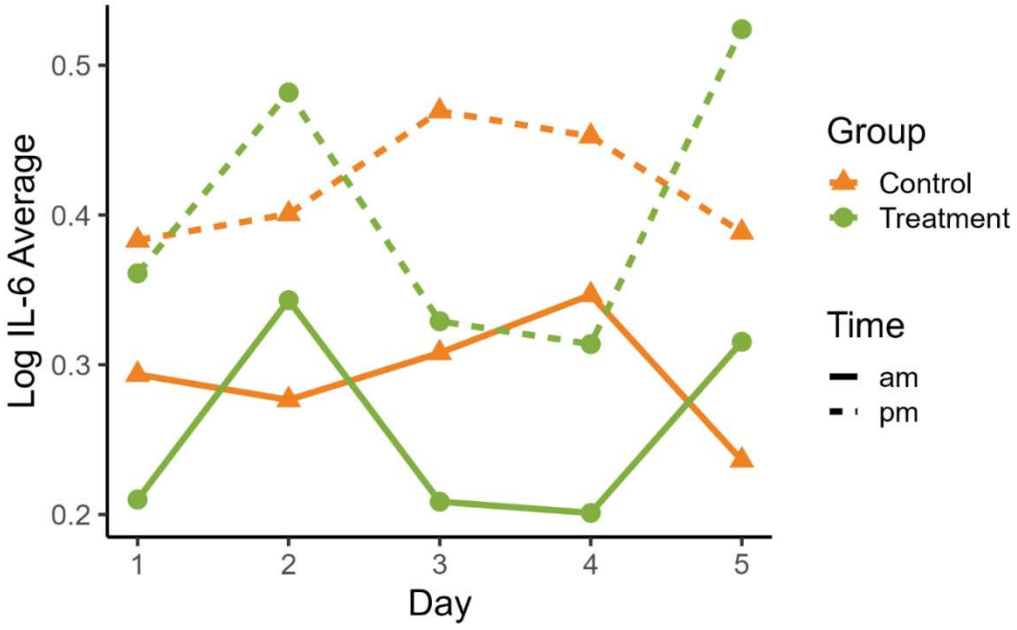



Figure 4.2: Average Log IL-6 across the five days for HTS Treatment (green) and NTS Control groups (orange) pre-shift (solid line) and post-shift (dotted line). Log IL-6 standard deviations ranged from 0.2811 to 0.9761

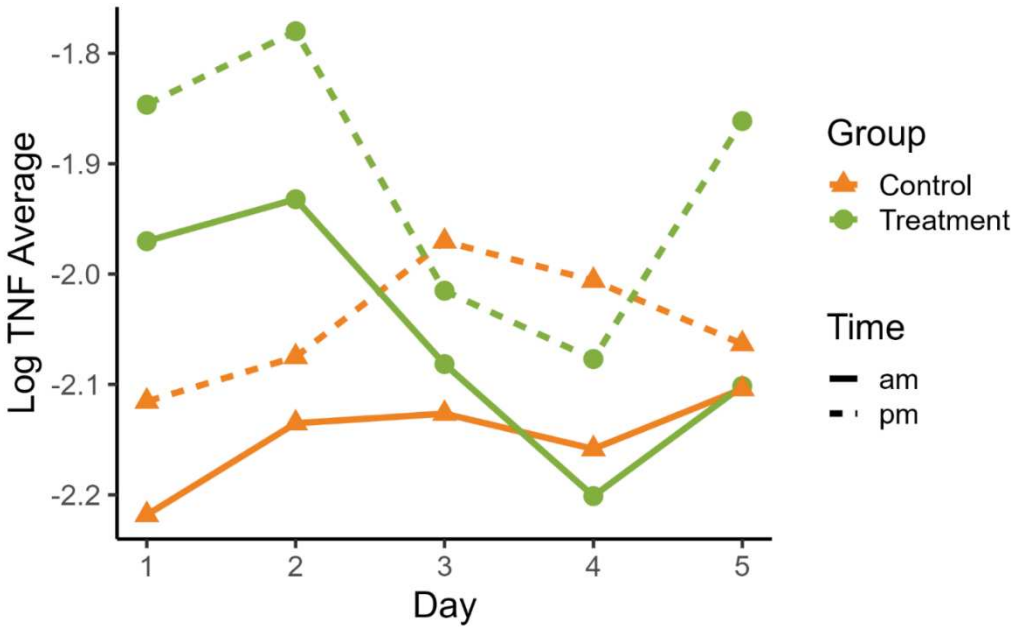



Figure 4.3: Average Log TNF-α across the five days for HTS Treatment (green) and NTS Control groups (orange) pre-shift (solid line) and post-shift (dotted line). Log TNF-α standard deviations ranged from 0.1233 to 0.6211.

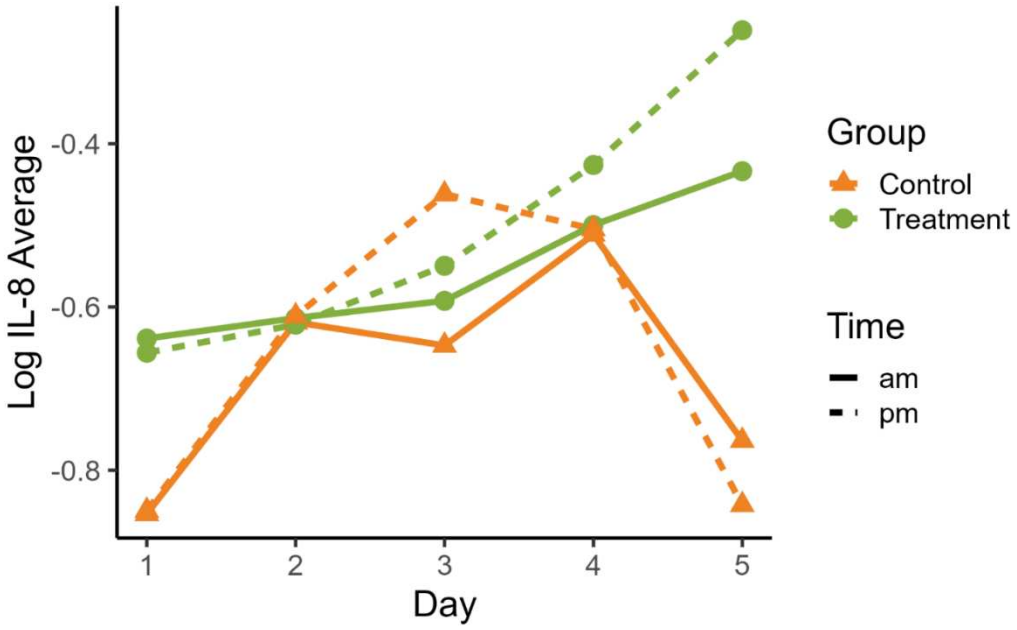



Figure 4.4: Average Log IL-8 across the five days for HTS Treatment (green) and NTS Control groups (orange) pre-shift (solid line) and post-shift (dotted line). Log IL-8 standard deviations ranged from 0.2217 to 0.6041

The dust model did not reveal sufficient evidence of an effect on average log mg/m$^3$ between treatment groups (HTS and control) (p=0.95), day (p=0.826), or an interaction effect between treatment and day (p=0.703). Similarly, the endotoxin model did not indicate evidence of an effect on average log EU/m$^3$ between treatment groups (p=0.781), among days (p=0.651), or an interaction effect between treatment and day (p=0.458). These results indicate similar dust and endotoxin exposure between treatment groups and sampling days. Therefore, exposure was not included as a variable in the biomarker model.

The biomarker model provided evidence of an interaction effect on average log concentrations between day and time of day for anti-inflammatory cytokine IL-10 (p=0.004). However, neither day nor time of day were significant main effects. To evaluate the interaction in absence of main effects, we created a plot of the model estimated marginal means shown if Figure 4.5.

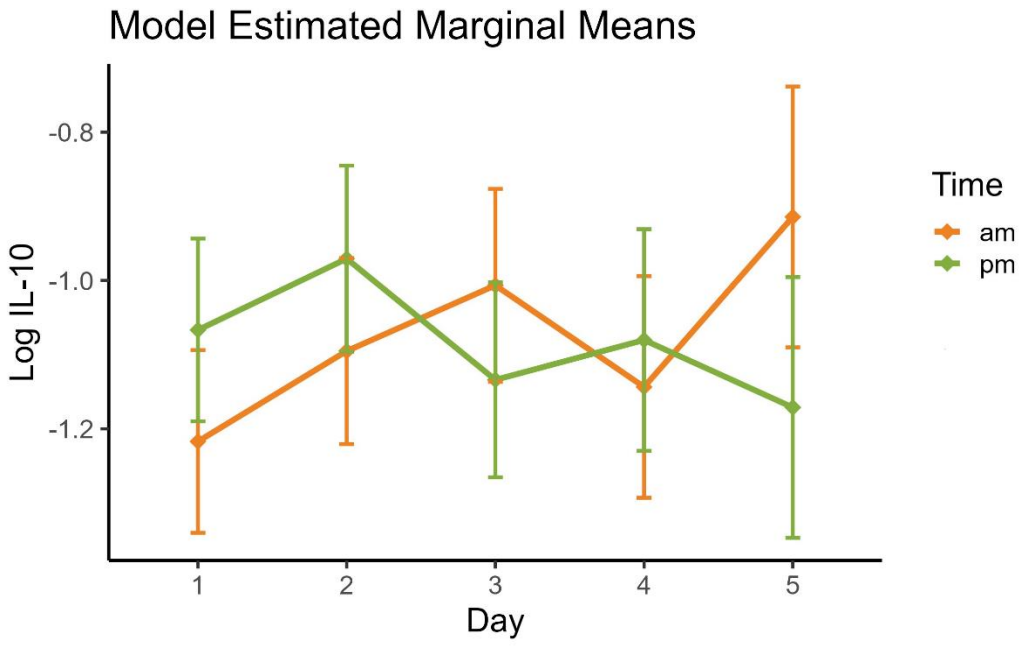



Figure 4.5: Model estimated marginal mean Log IL-10 concentrations across the five days for AM (orange) and PM (green) and associated confidence intervals

The plot illustrates a crossover interaction for IL-10 production between pre-shift (AM) and post-shift (PM) samples with AM samples exhibiting a positive trend while PM samples exhibit a negative trend across the 5-day sampling. There was no evidence of an interaction effect on average log concentrations between treatment, day, and time of day among all biomarkers. Further, there was not sufficient evidence of an interaction effect on average log concentrations between treatment and day or treatment and time among all biomarkers. Analysis of main effects provided evidence of differences in average log concentrations between time of day (AM and PM) for IL-13 ($p=0.047$), IL-1β ($p<0.001$), IL-6 ($p<0.001$), IL-8 ($p<0.001$), and TNF-α ($p=0.024$). Post-shift cytokine concentrations were higher than pre-shift cytokine concentrations for each significant result above. There was no evidence of differences in average log concentrations between treatment and control groups for any of the seven-modeled inflammatory biomarkers. There was no evidence of a difference in average log concentration between days among all biomarkers.

Microbiome diversity analyses revealed no significant differences in participant nasal microbiome alpha diversity, a measure of within sample diversity, between treatment and control groups. Beta diversity, a measure of between sample diversity, was not significantly different between group. There was evidence of differences in beta diversity between pre-shift and post-shift lavages ($p=0.002$) indicating a work shift related change in nasal microbiome. There was not sufficient evidence of differences in alpha diversity between pre and post shift. Additionally, there were not significant differences in alpha and beta diversity by day.

**Discussion**

Exposure to bioaerosols and associated inflammagens (e.g., endotoxin) present in the dairy environment is associated with an increased risk of upper and lower respiratory disease.

Past efforts to control dairy bioaerosol exposure have been mostly ineffective and cost prohibitive. In this study, pre- and post-shift hypertonic saline nasal lavages produced potentially advantageous albeit conflicting effects. Pre-shift pro-inflammatory cytokine concentrations were significantly lower than post-shift concentrations for IL-13, IL-1B, IL-6, IL-8, and TNF-α, confirming a work-related pro-inflammatory nasal response. Additionally, descriptive analysis of IL-10 anti-inflammatory concentrations shown in Figure 4.1 indicate an increasing anti-inflammatory response in both groups across the five-day sample period. However, no significant differences were observed in the model between treatment groups or between day among all biomarkers. Evidence of a significant interaction effect was found for IL-10 between day and time of day (p=0.004). Descriptive analysis of the model estimated marginal means (Figure 4.5) suggests a successful increase in log IL-10 across sample days among pre-shift samples while post-shift samples exhibit a downward trend. This crossover could be explained by IL-10s ability to auto regulate itself [49]. Essentially, upregulated pre-shift IL-10 may be auto regulating itself and inhibiting a large post-shift IL-10 response.

Our results illustrate a less robust immunomodulatory effect of hypertonic saline on anti-inflammatory cytokine production in comparison to existing literature [30–33]. We demonstrated increases in IL-10 over time at a descriptive level in both groups, indicating HTS and NTS may be capable of upregulating the anti-inflammatory response. Still, model results revealed no significant differences by group or day. In contrast, *in vitro* and *in vivo* testing of HTS was found to significantly increase IL-10 production in adult hemorrhagic trauma patients and produced a two-fold increase in peritoneal exudative macrophage IL-10 production compared to normotonic saline respectively [32]. The muted anti-inflammatory effect we observed could be due to study design limitations surrounding the intervention. Previous studies investigating HTS's anti-

inflammatory properties were able to test biomarkers at various intervals after administration allowing for quantitation of effects over time [32,34]. Our study was only capable of estimating transient changes in biomarkers due to collecting samples from lavages after administration.

For pro-inflammatory cytokines, there was no observed reduction in concentration attributable to the HTS treatment, contrary to our hypothesis. This result is a departure from our pilot study that measured significantly higher IL-6 concentrations in the HTS treatment group [19]. Earlier research found HTS treatment to be associated with significant decreases in TNF-a and IL-6 when compared to a normotonic saline treatment [32]. These studies were able to test lavage effects over time with multiple samples taken post-administration. Therefore, analysis of worker lavages as samples may be overlooking the true effect of the intervention on pro-inflammatory production. HTS can also illicit nasal mucosal irritation associated with transient increases in pro-inflammatory mediators. Analysis of post-administration lavages may be capturing this initial increase in inflammation while potentially missing subsequent decreases [35,50].

Dust and endotoxin exposure concentrations provided by full-shift personal breathing zone samples were well below suggested agricultural exposure guidelines and were lower than most measures found in previous dairy environments [6]. Unavoidable wet and cool weather conditions observed during sampling campaigns (snowstorm and ice storm) likely altered the dry dusty conditions regularly present in high plains dairy environments. Independent of cause, reduced bioaerosol exposure results in a less inflammagenic, cleaner environment and may be concealing the immunomodulatory effects of the HTS rinse.

When evaluating a workplace intervention, it is essential to consider potential side effects, barriers of adoption, and worker reception. The primary side effect of concern regarding frequent nasal irrigation is potential washout of healthy diverse microbial communities present in

the nose. Microbiome diversity analyses revealed that microbiome alpha and beta diversity did not significantly change after five consecutive days of the intervention. Moreover, the higher salinity of HTS lavages did not significantly shift nasal microbiome diversity compared to NTS lavages. Considering the low economic cost of hypertonic saline and the short amount of time to self-administer a nasal rinse (~10 sec), this intervention exhibits few adoption barriers in contrast to previous efforts [28,29]. Positive anecdotal comments made by participants also illustrate worker acceptability for this intervention.

It is important to interpret the results from this study in the context of its limitations and strengths. Foremost, our sample size (n=45) may not have provided the adequate power needed to estimate the effects of HTS treatment over the full five days. Although we more than quadrupled the sample size from the pilot study, only 18 participants were able to complete all five days due to variable work schedules. Additionally, workers taking medication for existing respiratory conditions and workers unable to meet respiratory testing requirements were excluded due to participation requirements possibly introducing healthy worker bias. This study was also limited by only measuring inflammatory markers in post-administration lavages to estimate intervention efficacy. Consequently, we were only able to measure transient changes in inflammatory markers. Additional measurement of inflammation at different intervals post-administration was not feasible in this study due to the nature of dairy operation schedules. The exclusion of other dairy bioaerosol inflammagens (i.e., peptidoglycans, bacterial pathogens, and viruses) and relevant pro-inflammatory mediators (i.e., histamine and leukotrienes) is another study limitation. Future investigations should expand exposure characterization and measure additional mediators to better estimate intervention efficacy. Finally, this study was limited by the absence of a true control group receiving no lavage. Future studies should include

participants receiving no rinse to elucidate whether the rinse itself provides protective effects regardless of tonicity.

## Conclusion

Regular bioaerosol exposure in the dairy environment and the resulting burden of respiratory disease in workers demonstrates the need for a low-cost, low-burden intervention. Pre- and post-shift administration of a hypertonic saline nasal lavage over five days was found to have limited but encouraging effect on the inflammatory response. When compared to controls receiving normotonic saline lavages, we observed no significant differences in inflammatory response in the HTS group. However, both groups experienced an increase in anti-inflammatory IL-10 concentration indicating the rinse itself may be more impactful than tonicity. Based on these results, it remains unclear if saline nasal rinses provide significant health benefits for bioaerosol exposed dairy workers. Therefore, we recommend future studies that measure inflammation incrementally post lavage and introduce a no-lavage control group to better estimate efficacy.

## Chapter 4 References


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# CHAPTER 5: EVALUATION OF PESTICIDE EXPOSURE ASSESSMENT STRATEGIES IN AGRICULTURE ADJACENT COMMUNITIES


## Summary

*Objective:* Misclassification of exposure to agricultural pesticides in agriculture-adjacent communities is a crucial limitation in efforts to understand the health effects of pesticides on residents. We aimed to evaluate agreement between three commonly used pesticide exposure assessment techniques: biomarkers, household dust, and GIS application data.

*Material and Methods:* Study participants were recruited from four communities identified to have elevated pesticide exposure in the Central Valley of California. Participating households were required <61 meters from fields in which pesticides were applied. Across two field campaigns in the summer and winter of 2019, we collected survey data, household dust, and urine from participants. Household dust was analyzed for the 11 most heavily applied organophosphate (OP) pesticides in the study area. Urine samples were analyzed for dialkyl phosphates, a group of six OP specific biomarkers. OP exposure was also estimated with the previously published OP pesticide application data from the California Pesticide Use Report (CPUR). To assess associations between exposure assessment techniques we ran Spearman rank correlation tests and constructed linear mixed models.

*Results:* Samples were collected from 80 participants across 28 households. Participants were primarily Hispanic/Latino (97.5%), 57.5% were female, and 70.6% of households had at least one resident who worked in agriculture. Correlation tests resulted in a moderate correlation between total OPs measured with the CPUR method and OPs in household dust (r=0.44). All other correlations were “negligible” (r=0.00-0.30). Linear mixed model results revealed no

significant associations between Urinary DAP concentrations and estimates from household dust or the CPUR method.

*Conclusions:* We found moderate to low correlations between OP pesticide exposure estimates using urinary DAPS, household dust, and the CPUR method. Studies only utilizing one assessment strategy may not provide an accurate exposure estimate.

## Introduction

Synthetic agricultural pesticides are broadly used in farming practices for their effectiveness in protecting crops from a wide range of harmful agents including weeds, insects, rodents, and fungi. As a result, pesticides have been instrumental in agricultural development due to resulting increases in yield, reductions of crop loss, and maintenance of food quality upon which ever-growing populations rely [1–3]. However, human exposure to agricultural pesticides has been linked to adverse health effects in both community and occupational environments. Occupational exposures to pesticides and resultant health outcomes, including mortality, have been well documented and have led to multiple recommended exposure limits [4]. Frequent exposure to high levels of pesticides found in occupational environments have made it easier to elucidate relationships between exposure and health effects. In community environments, where lower, chronic levels of exposure are observed, there is a mounting body of evidence that residents adjacent to agricultural land are exposed to a greater variety and amount of pesticides than the general population [5–10]. Elevated exposure in communities proximate to agricultural operations can be attributed to multiple sources, including pesticide spray drift [11,12], ingestion of contaminated produce and water[13–15], and tertiary exposure through occupationally-exposed household members [16,17]. Previous epidemiological studies investigating community pesticide exposure have proposed associations between residential proximity to agricultural operations and adult and childhood

cancers (e.g., breast cancer, leukemia, and brain tumors) [18–25], neurodegenerative diseases (e.g., Parkinson's and amyotrophic lateral sclerosis) [26–29], impaired neurodevelopment [30,31], birth outcomes (e.g., pre-term birth and neural tube defects) [32–35], and respiratory health (e.g., asthma, COPD, lung function) [36–38]. Yet, associations from these studies have largely been weak and in some cases demonstrate no effect conflicting with outcomes observed in similar studies [39–42]. For example, several studies have investigated either residential proximity to pesticide use or pesticide exposure and breast cancer with variable techniques[25,39,43–47]. Among them, multiple studies report a positive association [25,43,44] while others found no association [39,45–47].

Inconsistencies and weak associations found in existing literature may be attributed to uncontrolled confounding, ecological biases, and misclassification of exposure [5,48]. Specifically, misclassification of exposure has been identified as a crucial limitation in literature reviews attempting to understand the effects of agricultural pesticides use in surrounding residential communities [5,48]. Existing exposure assessment techniques used in occupational settings, such as personal samplers (e.g., OVS, IFV) utilize short sampling durations and are inadequate to capture the variable low-level exposures present in agricultural adjacent communities. Instead, previous studies have applied variable exposure assessment methodologies ranging from self-administered questionnaires to biomonitoring, agricultural pesticide use models, and household dust sampling [49–54]. Each strategy introduces a unique set of limitations and biases that contribute to potential misclassification.

The use of self-administered questionnaires to estimate pesticide exposure relies on participants' knowledge about household pesticide use, actual proximity to agricultural spraying, and recall of past spraying events which produce important sources of misclassification [55]. Biomonitoring of participant blood and urine for pesticide metabolites provides a quantifiable

measurement of exposure, but can be limited by metabolite specificity, high excretion rate, and short exposure windows [53,56,57]. For instance, dialkyl phosphate (DAP) metabolites, used to quantify organophosphate (OP) exposure, are excreted in just 6-24 hours post-exposure, detectable in food and non-agricultural environments, and are not specific to the pesticide from which they were derived [56,58,59]. Additionally, biomonitoring of pesticide metabolites does not provide information regarding specific sources but indicates total exposure from all sources. Analysis of household dust in carpeting can provide longer term exposure estimation from pesticides, but only captures particle phase pesticides that have infiltrated the house and requires existing carpeting in participant residences [60,61]. Lastly, geographic information system (GIS) agricultural use models leverage agricultural land use and pesticide use reports to estimate potential exposure in geocoded residential locations [49,62]. However, changes to surrounding land use, inadequate access to pesticide use reports, subjective selection of exposure window, and incorrect buffer zone identification can all limit exposure characterization and introduce misclassification [5,35,49]. Therefore, a more complete pesticide measurement technique that limits sources of misclassification is required to adequately estimate non-occupational exposure and inform epidemiologic models.

Refining our approach to pesticide exposure characterization is crucial to better recognize the factors that influence exposure and assess their impact on resident health. The objective of this study was to evaluate three exposure measurement techniques (household dust measurement, a group of six urinary metabolites, and GIS-based calculations) in communities adjacent to agricultural land within the Central Valley of California. Our goal is to understand the relationships between measurement strategies and determine if some combination provides a robust exposure

estimate. Study findings will help inform future community-based residential exposure assessment approaches.

## Materials and Methods

### *Study Area and Population*

The Study of Environmental Mixtures in Periurban Respiratory Outcomes (SEMIPRO) was conducted in Fresno and Tulare counties in California's Central Valley. These two counties sell more agricultural products than any others throughout the United States (National Agricultural Statistics Service). Consequently, Fresno and Tulare counties account for more than 20% of pesticide use across the state – leading to disproportionate exposure to pesticides among agricultural workers and residents (PUR). The agricultural workforce in Fresno and Tulare counties is primarily Latino and Spanish speaking, of which approximately 40% are migrant (National Agricultural Statistics Service). One of the primary goals of SEMIPRO was to establish a community-based cohort to estimate health effects of pesticides in this region.

Leveraging our partnership with the Central California Environmental Justice Network (CCEJN) in tandem with data from the California Pesticide Use Report (PUR), we were able to identify communities with elevated pesticide exposure in Fresno and Tulare counties. Enrollment criteria required participant households to be within 61 meters (200 feet) of an orchard in which pesticides were applied. Participants were recruited by CCEJN through door knocking campaigns and community-based events. Members of 200 households across four Fresno/Tulare County communities were contacted for study participation. Adults and children above the age of six living were invited to participate in the study.

*Pesticide Use Exposure Estimation.*

We estimated OP exposure from agricultural spraying operations based on household locations and 2018-2019 California Pesticide Use Report (CPUR) Data [63]. The PUR provides detailed data on the chemical type, amount (kg), and date of pesticide application by one square mile sections on a quarterly basis. We characterized exposure estimates for total OPs, chlorpyrifos, acephate, malathion, oxydemeton, dibrom, dimethoate, phosmet, diazinon, phorate, bensulide, and tribufos for comparison with household dust and urinary DAPs. Estimates were generated using the *purexposure* package [64] in R that leverages previously presented validated algorithms [49]. The *purexposure* package estimates exposure by calculating the percentage of land area in each section included in a circular buffer around geocoded residences, multiplying the kilograms of pesticide applied in each section by the fraction included in the buffer, summing the area weighted mass, and dividing by the buffer area. We calculated exposures for 500m and 1250m buffers around residences in 1 week, 1 month, 3 month, 6 month, and yearlong exposure windows prior to each participant's sample collection date.

*Sample Collection*

Survey and sample data were collected across two field campaigns in January and June 2019 to capture exposures during non-spraying and spraying periods, respectively. Households were surveyed and sampled once during each campaign. Participants completed questionnaires on health, household characteristics, demographics, pesticide use and exposure, and occupation. A full breakdown of questions included in participant surveys and their utility as an exposure assessment tool is presented in Kuiper et al. [65]. To assess household OP exposure, carpet dust was collected with a high-volume small surface sampler (CS3, Bend, OR) and sealed in a non-reactive container. All dust samples were collected according to the sampler specific operation manual [66].

Participants also provided same-day convenience urine samples to be analyzed for OP urinary metabolites after survey completion. Dust and urine samples were transported to California State University, Fresno, for processing and storage. Dust samples were removed from vacuum containers, wrapped in aluminum foil, and sealed in a ziplock bag. Urine and dust samples were then stored in a -80°C freezer until being shipped on dry ice to Colorado State University for analysis.

*Sample Analysis*

*Household Dust.*

A more detailed analytical methodology has been described previously [60]. Briefly, dust samples were sieved to separate and isolate dust particles <150 µm. Approximately 100mg of isolated dust was aliquoted from each sample. In cases where < 100mg was present, the total available mass was used. Using established methodology [67] and CSU Analytical Toxicology Laboratory study specific protocols, dust samples were analyzed for OPs. The eleven most heavily applied OP pesticides in the study area from 2011 to 2016 according to the PUR were selected for sample analysis: chlorpyrifos, acephate, malathion, oxydemeton, dibrom, dimethoate, phosmet, diazinon, phorate, bensulide, and tribufos. OPs were extracted from sieved dust into 1mL of acetonitrile through 30-minute sonication. Extracts were stored at -30°C for two hours to precipitate lipids, and then centrifuged. Samples were then vortexed with Agilent Universal d_SPE dispersive solid phase extraction sorbent ($C_{18}$, graphite, and magnesium sulfate) before being analyzed via liquid chromatography mass spectrometry (LC-MS/MS). Household dust free of target OPs was spiked and extracted to create controls and calibration standards. Samples were analyzed with an Agilent 1290 UH-PLC coupled with an Agilent 6460 triple quadruple mass spectrometer using an Agilent Jet Stream electrospray ionization source (Agilent, Santa Clara, CA,

USA). Linear regression with a six-point calibration curve from 5 to 500 ng/g was used to quantify samples. Each individual OP had a unique limit of quantitation (LOQ) associated with it. Individual OP concentrations below the limit of detection (LOD) for each sample were assigned a zero value. Concentrations above the LOD and below the LOQ were assigned a value equivalent to the LOQ divided by the square root of two. Total OP exposure was calculated as the sum of exposure to all eleven analyzed pesticides.

*Urinary DAPs.*

Urine samples were analyzed for the following DAPs: dimethyl phosphate (DMP), diethyl phosphate (DEP), dimethyl thiophosphate (DMTP), diethyl thiophosphate (DETP), dimethyl dithiophosphate (DMDTP), and diethyl dithiophosphate (DEDTP). Analysis followed established methods [68,69] and CSU Analytical Toxicology Laboratory study specific protocols. Three milliliter samples were transferred into glass test tubes containing 20 µL of internal standard (10 µg/mL each of DMP-D6, DMDTP-13C2, DETP-13C4, DEP-13C4, DEDTP-D10, and DMTP-D6) and stored at -80°C overnight. Frozen samples were lyophilized at -50°C with a Labconco FreeZone 4.5 lyophilizer. Samples were sonicated in 5mL of acetonitrile, mixed on a rotary mixer, and finally centrifuged. Supernatants were dried under nitrogen in a new test tube until volume was reduced to ~500 µL before being mixed with 1.5 mL of 0.1% formic acid in water and aliquoted into LC/MS autosampler vials for analysis. Samples were analyzed with an Agilent 1290 UH-PLC coupled with an Agilent 6460 triple quadruple mass spectrometer using an Agilent Jet Stream electrospray ionization source (Agilent, Santa Clara, CA, USA). Linear regression with a six-point calibration curve from 0.5 ng/g to 200 ng/mL was used for sample quantitation. Urinary DAP concentrations were normalized by creatinine concentration. Creatinine was quantified with a Creatinine

Colorimetric Kit (Cayman Chemical, Ann Arbor, MI). More detailed laboratory methods have been described in a previous publication [65].

*Data Management*

All data management and cleaning was conducted with R leveraging the 'tidyverse' package [70]. All household OP concentrations were reported in parts per billion (ppb). Each individual OP was analyzed independently and had a unique limit of quantitation (LOQ). In samples where an individual OP was not detected, a value of zero was imputed for that OP. Samples that had detectable levels of an OP below the LOQ were assigned a value equivalent to the LOQ divided by the square root of two. Total household dust OP concentrations were calculated by summing all eleven analyzed pesticides. All participants living in the same household were assigned the same household dust pesticide exposures due to dust samples representing the entire household. Total OP, chlorpyrifos and malathion concentrations in household dust were used in statistical models. All other individual OPs were not assessed in modeling steps due to detection rates lower than 50%.

Urinary DAP metabolite concentrations were estimated in ng/mL. As with household dust OP measures, non-detect samples were assigned a value of zero and detected samples below the LOQ were assigned a value equal to the LOQ divided by the square root of two. DAP concentrations were normalized by sample creatinine concentrations. All six DAPs were summed to estimate total DAPs (EDAP) in ng/mg creatinine. Subcategories of total dimethyl alkylphosphate (EDM) and total diethyl alkylphophates (EDE) were also calculated and presented in ng/mg creatinine. To account for the log normal distribution, urinary DAPs were log-transformed for statistical analyses. EDE was excluded from statistical modelling due to low

sample detection rate (<5%). Finally, the total dataset was subset to include only participants that had quantifiable urine, household dust, and PUR exposure estimates.

*Statistical Analysis*

Due to non-normal distributions in all three exposure estimates, we utilized nonparametric methods for initial comparison testing. We used Spearman rank correlation tests to evaluate associations between CPUR (total OPs, chlopyrifos and malathion), HH dust (total OPs, chlorpyrifos and malathion), and urinary DAPs (EDAP and EDM) exposure estimates.

We used linear regression to model log transformed concentrations of urinary EDAP and EDM. Separate linear mixed effect models were created for each summary DAP metric. Selection of independent variables included in the models was informed by a previously published investigation on factors associated with urinary DAP levels on this dataset [65]. Household and personal factors found to be significantly associated with urinary EDAP and EDM in the previous model were included in this model. Significant factors included in the base model were time living at current address, use of chemicals to control mice/rodents, age category (>18, 18-64, 65+), less than high school education, and seasonal employment status. A random effect for study participant nested within household was also included in the model to address repeat measures and clustering effects from participants living in the same household.

For the final model, we added total HH dust OP exposure estimates and total PUR OP exposure estimates to evaluate their predictive value in estimating urinary DAPs. For the PUR estimate, we chose to use the 1250 m buffer distance based on pesticide drift models [71]. We then ran both models iteratively at each PUR exposure window to determine which to include in the final model. Model estimates were consistent across exposure windows. Therefore, one-year 1250m PUR exposure estimates were selected for the final model to limit introduction of zeroes.

The importance of HH dust and PUR OP in each model was assessed using the AIC of the model before and after the addition of the OP exposure estimates.

Finally, using the base model that only included household and personal factors, we conducted a sensitivity analysis to assess the impact of individual HH dust OP concentrations on urinary EDAP and EDM. Chlorpyrifos and malathion were used for this analysis. All other individual OPs were excluded due to low detection rates (<50% of samples). Individual HH dust OPs were added to the base model and the importance of each was evaluated by measuring model AIC before and after their addition. A lower AIC after the addition of HH dust OPs was indicative of improvement in model fit.

## Results

*Descriptive findings*

Survey responses and urine samples were collected from 80 participants across 34 households during the January sampling campaign. In the follow up campaign in June, samples and responses were collected from 77 of the 80 original participants and 33 of the original 34 households. Across both campaigns, 157 surveys were administered, 154 urine samples were collected, and 52 dust samples were collected. Overall, 95 samples had survey responses, urinary metabolites, household dust, and PUR exposure estimates. Participant and household factors are presented in Table 5.1. A full table of household and individual factors can be found in Kuiper et al. [65].

**Table 5.1**: Household and individual characteristics

| | *Full Dataset (n = 80)* | | *Model Dataset (n = 58)* | |
|---|---|---|---|---|
| ***Individual Characteristics*** | ***n*** | ***%*** | ***n*** | ***%*** |
| Age Category | | | | |
| <18 years old | 10 | 12.5 | 7 | 12.1 |
| 18-64 years old | 57 | 71.2 | 39 | 67.2 |
| 65 years or older | 13 | 16.2 | 12 | 20.7 |
| Educational attainment | | | | |
| Less than high school | 42 | 52.5 | 28 | 48.3 |
| High school equivalent or greater | 28 | 35.0 | 23 | 39.7 |
| Employment status: seasonal | 10 | 12.5 | 6 | 10.3 |
| | ***Mean*** | ***SD*** | ***Mean*** | ***SD*** |
| Time spent living at current address | 12.8 | 9.4 | 13.4 | 9.7 |

| | *Full Dataset (n = 34)* | | *Model Dataset (n = 27)* | |
|---|---|---|---|---|
| ***Household Characteristics*** | ***n*** | ***%*** | ***n*** | ***%*** |
| Household use of chemicals to control rodents | 8 | 23.5 | 5 | 18.5 |

Urinary DAPS normalized by creatinine were detected in 44.3% (n= 66) of the 149 urine samples analyzed (Table 5.2). Dimethyl alkylphosphates were detected in 41.6% (n= 62) of samples while diethyl alkylphosphates were only detected in 4.7% (n = 7) of samples. Due to low detection rates, urinary EDE were excluded from downstream analyses. EDAP and EDM levels ranged from 0 to 332.6 ng/mg creatinine. EDE levels ranged from 0 to 29.5 ng/mg creatinine. Organophosphate pesticides were detected in 93.6% of household dust samples and total concentrations ranged from 0 to 651.7 ppb. OP pesticide exposure estimated from CPUR pesticide use data was detected for 95.5% of households and ranged from 0 to 191.3 kg/m$^2$. A full breakdown of exposure estimate descriptives are presented in Table 5.2.

**Table 5.2:** Summary statistics for urinary DAP concentrations (ng/mg creatinine), Household dust OP concentrations (ppb), and PUR OP concentrations ($kg/m^2$).

| Exposure Variable | *n* | Median | Minimum | IQR | Maximum | Rate of Detection |
|---|---|---|---|---|---|---|
| ***ng/mg creatinine*** | | | | | | ***% ($n_{detected}$)*** |
| EDE | 149 | 0 | 0 | 0 | 29.5 | 4.7 (7) |
| EDM | 149 | 0 | 0 | 5.2 | 332.6 | 41.6 (62) |
| EDAP | 149 | 0 | 0 | 6.2 | 332.6 | 44.3 (66) |
| ***ppb*** | | | | | | |
| HH OPs | 47 | 40.5 | 0 | 51.9 | 651.7 | 93.6 (44) |
| HH Chlorpyrifos | 48 | 22.8 | 0 | 48.6 | 395.3 | 79.2 (38) |
| HH Malathion | 50 | 3.5 | 0 | 4.9 | 29.1 | 76.0 (38) |
| HH Acephate | 48 | 0 | 0 | 14.1 | 575.4 | 45.8 (22) |
| HH Diazinon | 50 | 0 | 0 | 2.6 | 5.7 | 28.0 (14) |
| HH Dimethoate | 50 | 0 | 0 | 3.5 | 7.9 | 46.0 (23) |
| HH Oxydemeton-methyl | 50 | 0 | 0 | 0 | 313.6 | 4.0 (2) |
| HH Phosmet | 50 | 0 | 0 | 3.5 | 12.4 | 24.0 (12) |
| HH Tribufos | 49 | 0 | 0 | 3.5 | 48.9 | 26.5 (13) |
| HH Bensulide | 50 | 0 | 0 | 0 | 0 | 0 (0) |
| HH Dibrom | 50 | 0 | 0 | 0 | 0 | 0 (0) |
| HH Phorate | 50 | 0 | 0 | 0 | 0 | 0 (0) |
| ***$kg/m^2$*** | | | | | | |
| PUR OPs | 157 | 46.1 | 0 | 146.7 | 191.3 | 95.5 (150) |
| PUR Chlorpyrifos | 157 | 3.5 | 0 | 39.4 | 43.4 | 64.9 (102) |
| PUR Malathion | 157 | 0 | 0 | 9.2 | 39.3 | 35.7 (56) |

*Spearman rank correlations*

Figure 5.1 presents a heatmap of Spearman rank correlations between urinary DAP (EDAP and EDM), HH dust OP (HH total OPs, HH chlorpyrifos, HH malathion, and HH acephate), and PUR OP (PUR total OPs, PUR chlorpyrifos, PUR malathion, and PUR acephate) pesticide exposure metrics. Total HH dust OP concentrations and total PUR density of agricultural pesticide use estimates were moderately correlated ($\rho_s = 0.46$). Individual OPs chlorpyrifos and malathion measured in HH dust were each moderately correlated with their equivalent PUR density of use estimates (0.40 and 0.45 respectively). EDAP and EDM concentrations were not correlated with any of the other measurements included in correlation testing. Using shorter exposure windows

for PUR estimates did not improve correlations between measures. Correlations at shorter PUR exposure windows can be provided upon request.

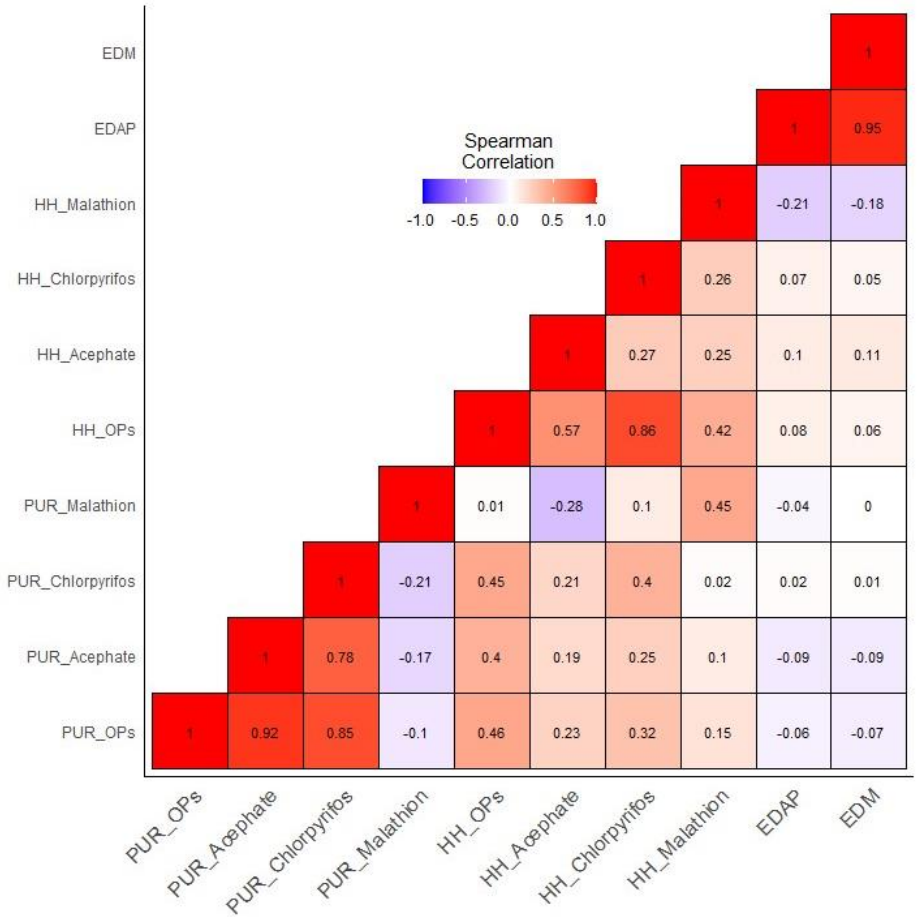



**Figure 5.1:** Heatmap of Spearman rank correlation coefficients between urinary DAPs, HH dust OPs, and PUR OPs.

*EDM model*

The final model for urinary EDM included variables previously reported as significant factors in Kuiper et al. associated with urinary DAPs in this study population, HH dust OP levels, and PUR use estimates (Table 5.3) [65]. Household and individual-level variables included in both models were time spent living at current address, household use of chemicals to control rodents, educational attainment, age category, visit month, HH dust total OPs, and PUR total OPs. One year increase in time spent living at their current address was associated with a 0.05 (95% CI: -

0.08 to -0.02) unit decrease in log transformed urinary EDM. Household use of chemicals to control rodents was associated with a 0.78 (95% CI: 0.04-1.44) unit increase in log-transformed urinary EDM compared to no household use. No other variables in the model were significantly associated with log transformed urinary EDM.

**Table 5.3:** EDM Model

| **Variable** | **Estimate** | **95% CI** | |
|---|---|---|---|
| ***EDM Model (n = 95)*** | | **Lower** | **Upper** |
| Time living at current address | **-0.05** | **-0.08** | **-0.02** |
| Household use of chemicals to control rodents[b] | **0.78** | **0.04** | **1.44** |
| Educational attainment | | | |
| Less than high school | 0.31 | -0.26 | 0.94 |
| High school equivalency and above[a] | -- | -- | -- |
| Age category | | | |
| 17 years or younger[a] | -- | -- | -- |
| 18-64 years old | 0.46 | -0.49 | 1.33 |
| 65 years or older | 1.11 | -0.08 | 2.25 |
| HH Dust Total OPs | 0.002 | -0.0003 | 0.003 |
| PUR Total OPs | -0.002 | -0.006 | 0.002 |
| Visit Month | | | |
| January[a] | -- | -- | -- |
| June | -0.09 | -0.49 | 0.36 |

**Bold** typeface indicates variables that were significant as determined by a 95 % CI that did not include the null. [a] Indicates referent level for indicator or categorical variables. [b] For indicator variables, the referent is the absence of the covariate/exposure for the participant (e.g., the referent for 'Household use of chemicals to control rodents' is no use)

*EDAP model*

The final model for urinary EDAP included variables previously reported as significant factors associated with urinary DAPs in this study population, HH dust OP levels, and PUR use estimates (Table 5.4). One year increase in time spent living at their current address was associated with a 0.05 (CI: -0.08 to -0.02) unit decrease in log transformed urinary EDM. Unlike the EDM model, household use of chemicals to control rodents was not significantly associated with log

transformed urinary EDAP. No other variables in the model were significantly associated with log transformed urinary EDM.

**Table 5.4:** EDAP Model

| **Variable** | **Estimate** | **95% CI** | |
|---|---|---|---|
| ***Total DAPs Model (n = 95)*** | | **Lower** | **Upper** |
| Time living at current address | **-0.05** | **-0.08** | **-0.01** |
| Household use of chemicals to control rodents[b] | 0.64 | -0.13 | 1.35 |
| Educational attainment | | | |
| Less than high school | 0.51 | -0.07 | 1.13 |
| High school equivalency and above[a] | -- | -- | -- |
| Age category | | | |
| 17 years or younger[a] | -- | -- | -- |
| 18-64 years old | 0.39 | -0.51 | 1.26 |
| 65 years or older | 0.80 | -0.37 | 1.93 |
| HH Dust Total OPs | 0.001 | -0.0006 | 0.003 |
| PUR Total OPs | -0.001 | -0.006 | 0.003 |
| Visit Month | | | |
| January[a] | -- | -- | -- |
| June | 0.02 | -0.42 | 0.53 |

**Bold** typeface indicates variables that were significant as determined by a 95 % CI that did not include the null. [a] Indicates referent level for indicator or categorical variables. [b] For indicator variables, the referent is the absence of the covariate/exposure for the participant (e.g., the referent for 'Household use of chemicals to control rodents' is no use)

*Individual HH OP sensitivity analysis*

When HH dust chlorpyrifos concentrations were added to the EDM model, there was a significant association between chlorpyrifos and log-transformed EDM (0.006; 95% CI: 0.001-0.011). There was also a significant association between chlorpyrifos and log-transformed EDAP (0.006; 95% CI: 0.001-0.011) when added to the EDAP base model. However, including chlorpyrifos did not improve the fit of either model. Instead, the addition of chlorpyrifos increased AIC by 7.2 in the EDM model and 7.3 in the EDAP model.

Including malathion HH dust concentrations in base EDM and EDAP models did not result in an improvement of fit. Additionally, there were no significant associations observed

between malathion concentrations and log-transformed urinary DAPs. All model estimates from sensitivity analyses are presented in Table 5.5.

**Table 5.5:** Model estimates for individual HH dust OP concentrations in sensitivity analyses.

| Model | OP pesticide | Estimate | 95% CI | | AIC Δ |
|---|---|---|---|---|---|
| | | | Lower | Upper | |
| EDM | Chlorpyrifos | **0.006** | **0.001** | **0.011** | **+ 7.3** |
| EDAP | Chlorpyrifos | **0.006** | **0.001** | **0.011** | **+ 7.2** |
| EDM | Malathion | -0.012 | -0.043 | 0.021 | + 7.9 |
| EDAP | Malathion | -0.015 | -0.049 | 0.019 | + 7.5 |

**Bold** typeface indicates variables that were significant as determined by a 95 % CI that did not include the null. [a]Full models contained the HH dust concentrations of OP indicated as well as covariates included in the base EDM and EDAP models.

## Discussion

Organophosphate pesticides are regularly used for agricultural applications on several different crops grown in the Central Valley of California. Residential exposure to this class of pesticides has been linked to various adverse health effects in non-occupational populations [18-38]. With increasing evidence of adverse health outcomes attributed to agricultural pesticide spraying in adjacent communities, optimal exposure assessment techniques need to be identified to promote meaningful epidemiologic inference. Inconsistencies and conflicting results from previous studies that estimate pesticide exposure and health impacts further demonstrate the limitations of currently utilized exposure assessment techniques. We sought to evaluate three exposure assessment techniques for OP pesticides among residents of agricultural adjacent communities.

In this study, we evaluated the relationships between OP exposures estimated with CPUR, HH dust, and urinary DAP assessment techniques. Our analyses found that none of the OP pesticide exposure estimates were highly correlated with one another and many exhibited low to zero correlation. There were moderate correlations observed between exposures estimated from

HH dust samples and CPUR for total OPs, chlorpyrifos, and malathion. This result is in line with correlations presented in previous studies comparing concentrations of OPs in HH dust and OPs estimated with CPUR [49,72]. However, urinary EDM and EDAP concentrations were not correlated with any of the other pesticide exposure measures. The lack of agreement could be due to the variable time windows each exposure assessment technique represents. DAPs are very short lived and can be excreted from the body with a matter of days [53,73], while OPs in HH dust can persist in the environment much longer with half-lives ranging from a week up to 120 days in soil [74,75]. PUR use estimates are reported on a weekly basis, but previous analyses, including our own, indicate that exposure windows of one year or greater provided similar or stronger correlations with other exposure assessment techniques [49]. The lack of homogeneity in the exposure duration each measure represents likely contributes to their lack of agreement.

EDAP and EDM model results support outcomes observed from the Spearman rank correlation tests. Neither HH dust OP measures nor PUR OP measures were associated with log normalized urinary DAPs concentrations. Additionally, model fit worsened with the addition of HH dust and PUR estimates. The lack of observed association between household dust and urinary DAPs supports results found in a previous publication investigating house dust and urinary DAPs in children [51]. Conversely, Tamaro et al. observed significant associations between household dust and urinary DAPs in an agricultural community for both children and adults [52]. These contradictory outcomes may be attributable to differences in specific OPs applied in the study area, model parameters, and study location. For example, in Tamaro et al. the primary OP pesticide applied azinphos-methyl was not in the top 11 OP pesticides applied in our study area. It is also possible that the relationship is more easily observable during peak application times which we were unable to capture during our sampling campaigns.

Sensitivity analyses revealed that HH dust chlorpyrifos levels were significantly associated with log transformed urinary DAPs. However, the effect estimate was quite small, and the AIC increased indicating model fit worsened with the addition of chlorpyrifos. This finding could be attributed to chlorpyrifos being the most detected individual OP; it is likely that while significant, the small change in urinary DAPs attributed to chlorpyrifos is not clinically meaningful.

Model estimates for household and individual characteristics evaluated in our previous publication [65] were similar to what we observed in this model. In both, household use of chemicals to control rodents provided the one of the largest effect estimates and was significantly associated with log transformed urinary DAPs. OP compounds are not approved for use in U.S. household rodenticides. Therefore, this finding is likely the result of an unmeasured confounder. In the EDM model, time living at the current address was also significantly associated with log-transformed urinary DAPs levels. Participants who reported living at their current address longer had lower urinary EDM levels. Residents who have spent more time in areas with agricultural pesticide spraying may engage in more risk reducing behaviors (e.g., shutting windows and remaining inside during and directly after spray events). This further illustrates the need for better assessment techniques that will capture confounders and provide a more complete profile of community pesticide exposure.

It should be noted that household and personal factors associated with pesticide exposure in previous studies were not found to be significant in this study and were excluded from our model. For instance, it is well documented that primary occupational exposure to pesticides (working in agriculture) can lead to elevated household pesticide levels through the "take-home" exposure route [10,67,76,77]. Further, in families living with someone working in agriculture, secondary occupational exposure (living with someone working in agriculture) has been found to

be associated with increased urinary DAPs concentrations [10,77]. Although primary and secondary occupational exposure wasn't found to be significant in our study population, seasonal employment in agriculture was associated with urinary DAPs in our previous publication and included in our model. Seasonally employed workers may perform job tasks that require more contact with pesticides than year-round workers. Additional research is necessary to identify underlying job tasks associated with higher occupational pesticide exposure to understand its contribution to community exposures.

Our study had several limitations, including small sample size. Removing participants without carpet dust samples exacerbated this limitation reducing our participant count from 157 to 95 but was necessary for modeling. Surface dust wipes were investigated as an alternative measure of household pesticide exposure in non-carpeted households, but detection rates were inadequate. This study was also limited by collecting one urine sample per field campaign. Repeated morning void samples are important to limit exposure misclassification for variable short lived urinary DAPs [76,78]. Without repeated samples collected over a short period of time it is possible that we missed what can be large variations in DAPs concentrations. Due to their high excretion rate and lack of explanatory power for accumulative effects, it has been posited that studies that report on urinary DAPs may lack clinical significance [56]. DAPs as a measure of pesticide exposure is further limited by the presence of preformed DAPs in food and environment, meaning urinary concentrations cannot be specifically attributed to OP exposure without environmental and dietary measurements [56]. Individual metabolites corresponding to one parent OP compound can be used alternatively, but don't provide the breadth of information on the OP class that DAPs can and have only been identified for a select few pesticides. In areas where multiple OPs are applied, like our study area, measuring DAPs provides a complete estimate of total OP exposure. Uncertainty

around the timing of specific spray events limited our ability to measure during known events. HH dust and PUR OP measures may correlate better with the short-lived DAPs if we conducted field campaigns during spray events. Additionally, generalizability of exposure assessment techniques evaluated here are limited to the OP chemical class and California. California is currently the only state with required pesticide use reporting restricting exposure estimation from use report data to one state. Further, a precipitous decline in OP use reported in California likely limits the applicability of the CPUR assessment technique for OPs and may signify a need to investigate new pesticides rapidly replacing OPs. In future research, we plan to address some of these limitations by utilizing larger sample sizes, collecting repeated morning void urine samples, and leveraging alternative sample types in the absence of carpet in households.

There were also several strengths of this study aimed at addressing challenges inherent to exposure assessment. The longitudinal study design provided multiple timepoints allowing us to evaluate the effect of season. The use of three exposure assessment techniques allowed for comparisons between multiple currently utilized strategies in the literature. Comparisons were further strengthened by integrating results from our previous evaluation of survey questions in exposure assessment. The in-person survey covered a variety of potential exposure sources at the individual and household level that informed our models.

**Conclusions**

Associations between community exposure to OP pesticides from proximate agricultural spraying and multiple adverse health outcomes demonstrate the value of accurate exposure assessment techniques. Comparisons of OP exposures estimated with HH dust, PUR use, and urinary DAPs OP assessment techniques, revealed moderate correlations between HH dust and PUR use estimates. Urinary DAPs were not correlated with HH dust or PUR use indicating a divide

between external and internal exposure assessment. Further, the lag time of pesticide reporting and lack of carpet dust limit the usefulness of the PUR and HH dust estimates. Connecting external exposure measures to internal exposure should be a primary focus for future studies evaluating OP exposure. Until we can determine better methods for estimating exposure, studies need to utilize robust techniques for both internal and external exposure measures.

**Future Research**

The research presented informs important recommendations for current and future characterizations of community pesticide exposure assessment. We recommend future efforts utilize both internal and external exposure assessment techniques to accurately measure potential sources and individual exposure respectively. Studies using metabolites also found in food and environment like DAPs should include dietary and environmental measures to parse out proportions attributable to pesticide exposure. We also recommend that studies measure household and individual factors previously found to be associated with pesticide exposure and identify new variables that help relate internal and external exposure (e.g., weather conditions, household cooling strategies, time at address, primary pesticide exposure, etc.). As assessment techniques continue to be refined, subsequent efforts towards standardization of methodology should be made to improve comparability between studies lacking in current literature.

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# CHAPTER 6: CONCLUSIONS

The agricultural industry worldwide provides the public with food, employment, and represents a significant proportion of the economy. Agriculture is also documented as one of the most hazardous industries for workers and relies on a vulnerable migrant workforce to meet ever growing demand. Among the diverse hazards present in agricultural operations inhalation hazards have been identified as a primary concern and are specifically mentioned in NORA. Agricultural inhalation exposures have been shown to illicit a diverse range of adverse symptoms and health outcomes in exposed individuals. The research presented here supplements and informs gaps in the literature on exposure assessment and intervention of agricultural inhalation hazards in occupational and community environments. Aims 1 and 2 sought to address a lack of viable interventions for bioaerosol exposed dairy workers by evaluating the efficacy of a novel low-cost nasal lavage in reducing worker respiratory inflammation. Aim 3 investigated concerns regarding the accuracy of OP pesticide exposure assessment techniques used in agriculture adjacent communities by comparing three techniques frequently used in literature.

## Chapter 3

A novel low-cost hypertonic saline nasal lavage intervention was first piloted in chapter 3. Results from the pilot study indicated that treatment group workers receiving hypertonic saline nasal rinses produced significantly higher concentrations of IL-10 (anti-inflammatory cytokine) than the control group receiving normotonic saline ($p < 0.01$). Additionally, the treatment group experienced an increase in IL-10 across the five-day study period. Significantly higher concentrations of IL-6 and IL-8 pro-inflammatory markers were also observed in the treatment group calling intervention efficacy into question.

**Chapter 4**

In chapter 4, we conducted a more thorough evaluation of hypertonic saline nasal lavages as an intervention in a larger cohort. Study design changes were made to include nasal microbiome diversity metrics and to give treatment group participants hypertonic saline rinses pre- and post-shift. Results indicated no significant differences in pro or anti-inflammatory cytokines between treatment group or day. Additionally, no evidence of washout effects from the rinses was found as worker microbial diversity metrics did not significantly shift between group or day. Interestingly, both groups experienced non-significant increases in anti-inflammatory IL-10 concentrations across the five-day study period.

Overall, the viability of saline nasal rinses as an intervention in dairy environments remains unclear. Despite increases in anti-inflammatory cytokine concentrations observed in the treatment group of both studies, conflicting pro-inflammatory increases in the pilot and non-significant increases in the main study complicate interpretation. Further, increases in anti-inflammatory cytokines among both groups in the main study indicate rinses may be effective regardless of tonicity. Ambiguous results may be attributable to limitations in study design necessitated by the variable nature of dairy operations that only allowed for measurement of transient changes in inflammation from collected lavages. Incremental measurement of inflammation post-lavage could provide the added context needed to fully evaluate intervention efficacy.

**Chapter 5**

Relationships between OP pesticide exposures in agricultural adjacent communities estimated from three assessment techniques used in the literature were explored in chapter four. Moderate positive correlations were observed between household dust exposure estimates and

pesticide use model exposure estimates mirroring correlations previously published. Conversely, exposure estimates from urinary DAP metabolites were not correlated with household dust or use model estimates. Linear mixed models confirmed correlation test results as no significant associations were observed between urinary metabolite concentrations and estimates from household dust or the use model.

Given the observed lack of association between the three OP pesticide exposure assessment techniques, measures of external exposure (household dust and GIS methods) do not appear to provide an accurate measure of internal exposure (Urinary DAPs). Instead, it seems that each technique provides a unique set of information relating to exposure. Use models provide an accurate estimate of potential external exposure specific to agricultural applications, household dust concentrations estimate indoor exposure to particle phase pesticides from all sources (e.g., dietary, household products, and agricultural spraying), and urinary DAPs estimate total internal exposure from all sources in addition to DAPs present in the environment. Therefore, previous research on community pesticide exposure relating an external exposure measure to internal exposure and vice versa should be interpreted carefully.

**Future Work**

The findings presented here should inform future research exploring assessment and intervention of agricultural inhalation exposures. Based on encouraging anti-inflammatory effects reported in chapters 3 and 4, further investigation of hypertonic saline nasal rinses is warranted. To address limitations we experienced, future work should measure inflammation incrementally post lavage to better understand intervention effects over time. Introduction of a true control group that receives no lavage should also be implemented in future efforts to evaluate the impact of the rinse independent of tonicity. Future studies on community pesticide

exposures should identify new variables and factors that can relate external and internal exposure measures. Until we identify the underlying mechanisms that explain disagreement between measures, future exposure assessments should include internal and external measures as well as household and individual factors previously associated with exposure.