Measures of Dust, Endotoxin and Exhaled Nitric Oxide among Dairy Farm Workers

Final Progress Report

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List of Terms and Abbreviations

eNO – Exhaled Nitric Oxide

NO – Nitric Oxide

FEV_1 – Forced Exhaled Volume in 1 second

FVC – Forced Vital Capacity

ppb – parts per billion

ATS – American Thoracic Society

COPD – Chronic Obstructive Pulmonary Disease

HP – Hypersensitivity Pneumonitis

Abstract

Inhalation of organic dust including endotoxin has been associated with inflammatory response of the pulmonary system. Limited studies have evaluated the work shift effects of endotoxin on respiratory outcomes for workers in the dairy industry, such as spirometry changes. Additionally, measurement techniques for exhaled nitric oxide (eNO) have been standardized by the American Thoracic Society (ATS) and used as a biomarker to identify diseases marked with lung inflammation. Dairy parlor workers are known to work long hours in one location with little task variability. The objectives of this study were to quantify exposure concentrations of inhalable dust and endotoxin among dairy parlor workers, evaluate acute cross-shift changes in respiratory status using spirometry, and assess the effectiveness of exhaled nitric oxide for detecting cross-shift bronchial responsiveness changes.

Sixty-two dairy parlor workers from 10 large herd dairy farms across Iowa, Minnesota, Wisconsin, and South Dakota were recruited into the cross-sectional study. A total of 160 measures of spirometry, exhaled nitric oxide, and pulmonary symptoms were collected. Additionally, 160 inhalable aerosol exposure samples using personal breathing zone sampling were collected among dairy parlor workers. Aerosol exposure samples were also analyzed for dust, and endotoxin and muramic acid.

Inhalable dust concentrations ranged from 0.09 – 4.95 mg/m^3 with a geometric mean of 0.58 mg/m^3. Inhalable endotoxin concentrations ranged from 4-1968 EU/m^3 with a geometric mean of 118 EU/m^3. Inhalable muramic acid concentrations ranged from 0.802-41.0 ng/m^3 with a geometric mean of 3.59 ng/m^3. The study group's pre-shift forced expiratory volume in the first second (FEV_1) as a percentage of predicted was an average of 93.4%. Study group cross-shift FEV_1 decreased by -1.16%. Six participants with moderate post-shift concentrations of eNO had an average FEV_1 cross-shift change of -3.19%. Dairy parlor workers are exposed to lower concentrations of dust than has been observed in previous studies. However, some workers may still be adversely impacted. Future studies should test simple, effective, interventions in milking parlors to reduce dust exposure among dairy parlor workers.

Section 1

Significant Findings

Under Aim 1, we measured exhaled nitric oxide using the NIOX Mino among dairy parlor workers before and after their work shift. The NIOX Mino was easy to use on the farm and the participants understood instructions (**Fig. 1-1**). The NIOX Mino indicates to the study subjects when to inhale and exhale using an audio/visual display. Data were easily collected and recorded from the NIOX Mino in units of parts per billion (ppb) (**Fig. 1-2**). We observed a mean change of -1.4 ppb (SD 4.4) across the work-shift, over the course of the study. This observed difference was not statistically significant and few measures of exhaled nitric oxide were considered "high" based on previously published population based data. However, the NIOX Mino performed as designed with minimal difficulties. Recruitment was a challenge for this study as we focused the study on one working group at the dairy (i.e., dairy parlor workers). We chose these groups as their daily tasks are similar across working days of the week and season (i.e., milking and moving cows). To achieve the timeline of the project we returned to farms that we had previously visited and allowed dairy parlor workers who had previously participated, to participate again if interested. At the completion of the study we had enrolled 62 dairy parlor workers and collected measures during across eighty work shifts to achieve the proposed sample size.

Fig. 1-1. Measurement of exhaled nitric oxide before the work shift.

Under Aim 2, we measured occupational exposure among 62 dairy parlor workers and returned and repeat sampled 18 of the 62 workers to complete the project.

Occupational exposures were measured in the breathing zone of the workers and included inhalable aerosols such as dust, endotoxin, and muramic acid. Inhalable dust concentrations ranged from 0.090 – 5.0 mg/m^3 with a geometric mean of 0.58 mg/m^3. Inhalable endotoxin concentrations ranged from 4-1968 EU/m^3 with a geometric mean of 118 EU/m^3. Inhalable muramic acid concentrations ranged from 0.802-41.0 ng/m^3 with a geometric mean of 3.59 ng/m^3 (Figure 1-3)

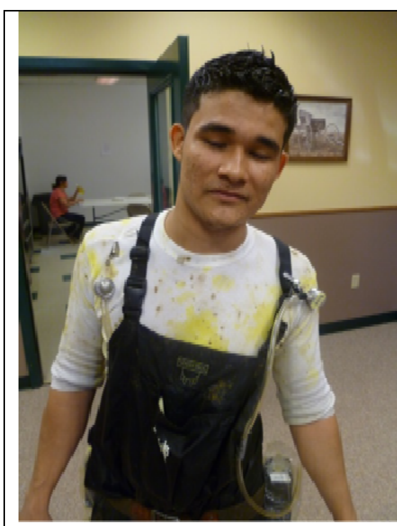

Fig. 1-3 Occupational exposure assessment in the breathing zone of the parlor worker.

Fig. 1-2. NIOX Mino indicates when study subjects should inhale and exhale using an audio/visual display.

Under Aim 3, we collected both pre and post shift questionnaires assessing pulmonary symptoms. Of the 62 participants, 90% were male and 94% were hispanic/latino which was consistent with previous studies. Approximately 16% of participants reported having at least one of the following symptoms after work: cough, wheezing or shortness of breath.

Translation of Findings

The NIOX Mino was easy to use in the farm setting. This device has the potential to offer an immediate and minimally invasive measure of lung inflammation. However, challenges may exist when using this device to detect low levels of lung inflammation. The inhalable exposures in this study of dairy parlor workers were relatively low compared to other studies of agricultural dust exposure.

We have disseminated our work at national conferences. Two peer review publications are currently in preparation. Furthermore, this work has been translated and presented to the International Dairy Research Consortium organized by Colorado State University

Outcomes/ Impact

Our contribution is significant for the following reasons: 1.) We measured inhalation exposure using a task-based approach to further characterize inhalation exposure among dairy farm workers, specifically dairy parlor workers. This project is important as our exposure assessments were focused on parlor workers and most if not all previous dairy work has combined exposure estimates for all dairy workers, offering little information to develop exposure interventions. Dust exposures during dairy parlor work are lower than what has been reported among other farm workers. This observation, will allow us to further explore where aerosol exposures occur on dairy farms and look for low-cost engineering solutions. 2.) Collecting eNO measures among dairy farm workers was easily performed and this tool could be applied to other farm workers. Challenges exist with interpreting individual measurements to existing population based data; therefore, pre/post comparisons may be the best approach for using this tool. 3.) Dairy farms are becoming larger due to economies of scale. Occupational health and safety is a growing concern for farm owners as their workforce becomes larger, is often from countries other than the United States and non-English speaking. Furthermore, dairy farm work continues for 24 hours a day, seven days a week, leading to more than 40 hours in a working week. Continued work to evaluate and develop business sensitive solutions to occupational health and safety challenges on dairy farms is needed.

Our findings also provided avenues for future research, which we are now pursuing as part of an R01 Award through NIOSH. We have identified several areas where both industrial hygiene and ergonomic engineering controls can be implemented on dairy farms. A programmatic approach using these controls in combination with a focus on Total Worker Health may lead to a reduction in occupational injury and illness as well as increased wellness on dairy farms.

Section 2

Background

Agricultural workers are an underserved population with a high rate of occupational fatalities, injuries, and illnesses, and limited resources for prevention. Lung disease among agricultural workers has been recognized for some time, and current estimates are that nearly 1,000,000 agricultural workers are at risk for lung disease. Respiratory diseases often identified among agricultural workers include chronic bronchitis, asthma, byssinosis, Chronic Obstructive Pulmonary Disease (COPD), hypersensitivity pneumoninitis (HP), Silo Filler's Disease and episodes of organic dust toxic syndrome (ODTS). Over the last 30 years, research on the respiratory health of agricultural workers has largely been dedicated to CAFO workers, particularly in the swine industry. Respiratory disease and symptoms most often reported among CAFO workers include: chronic bronchitis, asthma, wheeze, dyspnea, chest tightness, dry cough, short-term reduction in lung function, and ODTS. Numerous exposure assessment studies have been performed among swine CAFO workers, and potential engineering controls have been evaluated. The American Conference of Governmental Industrial Hygienists (ACGIH) promulgates a threshold limit value (TLVs) of 4 mg/m^3 total dust value for grain dust (ACGIH, 2013). Therefore, other agricultural dusts (e.g. agricultural dust from dairy facilities) fall under guidelines established for "particulates not otherwise classified" which have a total-dust TLV of 10 mg/m^3 (ACGIH 2013). Furthermore, this TLV is high relative to a proposed guideline of 2.4 mg/m^3 for total dust that has been recommended to prevent adverse work-related health effects in swine and poultry production environments (Donham 1995). Establishing total dust guidelines specific to agricultural facilities is complicated the fact that the ACGIH TLV committee intends to replace the existing total dust TLVs with inhalable, thoracic, and respirable particulate TLVs (ACGIH 2013). Therefore, future studies of dust exposure in agricultural environments should include a measure of inhalable particulate (Reynolds, 2009).

Similar to swine CAFO workers, studies of dairy farm workers have shown increased rates of chronic bronchitis, and occupational asthma. Furthermore, some studies have reported decreased pulmonary function among dairy farmers, along with pulmonary symptoms (e.g. wheezing, cough). This decreased lung function suggests some form of pulmonary inflammation or the onset of obstructive lung disease. Previous work by Dr. Reynolds has also found lower baseline FEV_1 and significant cross-shift decrements in FEV_1 and FVC among Colorado Dairy workers. These data suggest that dairy farm workers are at risk for pulmonary inflammation or disease.

Researchers have expressed a need for an inexpensive, easy to use, non-invasive measure of lung inflammation. Other markers of inflammation such as peripheral blood and a nasal lavage can be uncomfortable for study participants, and the analyses of both blood and nasal lavage are expensive and time consuming. Upon further investigation, eNO was identified as a potential solution.

NO is a radical gas that diffuses freely from the site of production in the lung. NO is produced by NO synthases in various cell types, including inflammatory cells in the human lung. NO is responsible for neurotransmission, vasodilatation, and immune enhancement in the lung. However, increased levels of NO can lead to inflammatory activity in the lung. Specifically, the presence of NO increases the genetic expression of proinflammatory cytokines, which, in-turn, increases the production of these cytokines in the lung (e.g. interleukin – 8) (Sparkman 2004).

Standardized methods have been established to measure eNO by the American Thoracic Society (ATS) and the European Respiratory Society (ATS/ERS 2005). Concentrations of exhaled NO have been shown to correlate with airway inflammation in diseases such as adult respiratory distress syndrome and childhood asthma. eNO is considered a reliable non-invasive marker for eosinophilic airway inflammation (e.g. asthma, hayfever). Additionally, measuring concentrations of eNO has been suggested as a screening tool to detect early stage or "active" COPD. Previous studies have reported eNO values for the general population ranging from 3 ppb – 39 ppb of NO. These data are difficult to interpret as different eNO analyzers and exhalation rates were used and some of the subjects included in these studies may have been undiagnosed asthmatics.

Agents which may contribute to pulmonary inflammation and have been identified among dairy farm operations include agricultural dust, endotoxin and muramic acid. Agricultural dust from livestock facilities, often referred to as organic dust, can be a complex mixture of hair, animal feed, animal bedding, insects, animal skin, animal feces, fungal mycotoxins, and bacterial endotoxins. Additionally, agricultural dust can have a significant proportion (10–20 %) of inorganic material such as clay and silica. Given the complex mixture in agricultural dusts, inflammatory mechanisms resulting from occupational exposure to agricultural dust are complex, often exhibiting characteristics of chronic bronchitis, bronchial hyper-responsiveness, asthma, and COPD. Furthermore, exposure to endotoxin results in lung inflammation leading to respiratory symptoms and decreased pulmonary function. Inhalation exposure to endotoxin induces inflammatory cell activation, the release of inflammatory mediators (interleukins) which results in subsequent damage to lung tissue. Endotoxin exposure also results in respiratory symptoms, decreased pulmonary function and increased asthma severity. Furthermore, changes in NO, eNO and the genetic expression of enzymes related to the production of NO (NO synthases) have been reported among healthy individuals and animals after experimental exposure to endotoxin. Therefore, eNO would appear to be a useful indicator of endotoxin exposure among workers who may be occupationally exposed to endotoxin (e.g., dairy farm workers).

Asthma, HP, as well as forms of COPD (e.g. chronic bronchitis) have been identified as being more prevalent among agricultural workers, particularly dairy farmers. Therefore, measuring the concentration of eNO among these large herd dairy farm workers may be useful in detecting early lung disease among these workers. eNO has been evaluated among individuals diagnosed with underlying COPD with positive, negative and positive correlations observed. However, eNO may be useful in monitoring COPD as several studies have identified elevated eNO among patients with exacerbated or severe COPD compared to patients with stable COPD. Furthermore, eNO was used to assess asthmatic airway inflammation during inhalation challenges to occupational agents in patients with suspected occupational asthma. Piipari et al. found that in patients with normal or slightly elevated basal eNO and late bronchoconstriction, significant change in eNO was observed. However, in patients with high baseline eNO, no change in eNO was observed with patients who had significant bronchoconstriction. Given these observations, care must be taken when interpreting changes in eNO. Other uses of eNO include assessing the effectiveness of an educational intervention among farmers who were diagnosed with occupational asthma (Dressel 2007). Dressel et al reported a decrease in eNO among farmers who were diagnosed with occupational asthma, compared to controls that were not provided the intervention. Clearly, eNO is a useful tool for the diagnosis, evaluation of treatment effectiveness, and interventions involving underlying obstructive occupational lung disease. However, more information is needed to determine the extent to which eNO can be used to evaluate occupational lung disease.

Specific Aims

As written in the original proposal, the specific aims of the K01 were:

Specific Aim 1: Measure the concentration of exhaled nitric oxide levels among a sample of large herd dairy farm workers before and after an 8 – hour work shift (N=80).

Specific Aim 2: Determine the occupational exposure to dust and endotoxin among a sample of large herd dairy farm workers (N=80).

Hypothesis 1: Cross-shift changes in exhaled NO and FEV_1 and FVC will be explained by increased exposure to dust and endotoxin.

Specific Aim 3: Determine prevalent pulmonary symptoms among a sample of large herd dairy farm workers both before and after an 8 – hour work shift (N=80).

Hypothesis 2: Large herd dairy farm workers will report more pulmonary symptoms post shift, compared to pre-shift.

Methodology, Results, and Discussion

Modified aims and hypothesis:

Specific Aim 1: Measure exhaled nitric oxide and pulmonary function changes across the work shift of dairy parlor workers (N=80).

Specific Aim 2: Determine the occupational exposure to dust, endotoxin and muramic acid among a sample of dairy parlor workers (N=80).

Hypothesis 1: Cross-shift changes in exhaled NO and FEV_1 and FVC will be explained by increased exposure to dust, endotoxin and muramic acid.

Specific Aim 3: Determine prevalent pulmonary symptoms among a sample of large herd dairy farm workers both before and after an 8 – hour work shift (N=80).

Hypothesis 2: Large herd dairy farm workers will report more pulmonary symptoms post shift, compared to pre-shift.

Study Sample Population

Dairy parlor workers in Iowa, Minnesota, Wisconsin, and South Dakota were recruited into the cross-sectional study. A total of 9 dairy farms were used to recruit participants into the study, eight of the farms having a herd size of over 1000 cows. A total of 62 dairy parlor workers classified as milkers or pushers were recruited. Being a “pusher” is a job classification on the dairy farm where workers move or “push” cows within, to and from the milking parlor. The study was approved by the University of Iowa Institutional Review Board and data were collected from May 2012 to January 2013. Dairy workers were eligible for voluntary participation in the study if they were between the ages of 18-67, , non-smokers, worked in the dairy parlor as a milker or pusher, were willing to perform the pulmonary function testing, participate in exposure assessment activities, and answer a pulmonary symptom questionnaire. The questionnaire was modeled after the American Thoracic Society (ATS) pulmonary symptom questionnaire and administered to participants pre-shift and post-shift. The questionnaire collected information including demographics, working characteristics, and symptoms related to acute organic dust responses.

Environmental Sampling

Personal sampling for inhalable aerosols was performed on all parlor workers. Aerosols were sampled with Button Aerosol Samplers (SKC, Eighty Four, PA) which collects aerosol particles less than 100 microns and follows the ACGIH criteria for inhalable particulate sampling. Samplers were placed on both the left and right shoulder, within the participant’s breathing zone. Air was collected using personal sampling pumps (SKC AirChek XR5000, Eighty Four, PA) at a calibrated flow rate of 4 Liters per minute. Aerosols were collected using pre-weighed polyvinyl chloride filters (SKC, Inc., Eighty Four, PA; 5.0 µm pore size; 25 mm diameter) for gravimetric, endotoxin and muramic acid analysis. After sample collection, samples were refrigerated until we returned to the laboratory.

Upon returning to the laboratory, exposure samples were placed in a desiccating chamber for a minimum of 24 hours to remove excess water. After desiccation the filters were allowed to equilibrate to room humidity for 12-hours prior to post-weighting. After gravimetric analysis the filters were individually placed in sterile tubes and transferred to a -20° Celsius freezer until endotoxin and muramic acid analysis.

Endotoxin

Exposure samples were shipped to a collaborating laboratory for endotoxin analysis using a Recombinant Factor C Endotoxin Assay (Cambrex, East Rutherford, NJ). Samples were extracted using sterile, pyrogen-free water with 0.05% Tween-20 for 1-hour at 22° Celsius while shaking the sample continuously. Samples were then added to a 96-well plate with a 100 microliter mixture of enzyme, buffer, and fluorogenic substrate. Afterwards, the plate was incubated at 37° Celsius for one hour before analyzed using a fluorescence microtiter plate reader (Biotek Instruments FLX800TBIE) with Excitation/Emission 380/440nm. Background fluorescence of 0 EU per milliliter was subtracted and the log-transformed values of the fluorescence change were compared to log endotoxin concentrations. Four assay reagent blank wells were used as reference and controls for the pyrogen-free status of the reagent water, centrifuge tubes, pipette tips, and microplates. Quality assurances with endotoxin spiking assays were performed to analyze matrix interference or enhancement.

Muramic Acid

The muramic acid analysis has been used in other published papers by Reynolds et al, and Poole et al. The same procedure was used to analyze aerosol exposure samples in this study. The procedure is as follows. To prepare samples for GC/MS/ MS analysis of muramic acid (marker of PGN), lyophilized samples and standards (0, 2, 5, 10, 50, 100, or 500 ng) were digested in 1 ml of methanolic HCl overnight at 100ºC. A solution of C13 muramic acid as an isotope dilution internal standard was obtained by digestion of 4mg of C13-labeled algal cells (99% C13) as for the samples. Prior to SPE, 30 ul of the C13 muramic acid solution was spiked into each sample and standard as an internal standard. SPE of the sample was done with Strata-XC 60mg/3mL strong cation exchange columns conditioned with 2 ml of methanol and 2 ml of aqueous 0.1% H3PO4. Samples were loaded to the column with vacuum assistance and then dried under full vacuum for 20 min. Muramic acid was eluted from the column with 5% NH4OH in acetone. Ten ul of 100ug/ml pentadecanol was added to the eluent and then dried under a stream of nitrogen. Dried samples were incubated with 50ul BSTFA/1%TMCS and 5ul pyridine at 85ºC for 30 min to form trimethylsilyl derivatives. Following derivatization, cooled samples were diluted to 100ul with heptane for GC/MS/MS analysis.

Muramic acid was analyzed with an oven temperature profile of 120 to 290ºC at 20ºC/min, holding 280ºC for 4 min. The inlet temperature was 260ºC and the GC-mass spectrometer interface temperature was set at 300ºC. The mass spectrometer was operated in MRM mode with fragment ions generated with collision energy of 6eV. The two MRM transitions monitored for muramic acid were m/z 185>142 and 185>130 and m/z 190>145 for C13 muramic acid. Results are presented in ng muramic acid per mg dust.

Spirometry

Lung function tests were administered by trained technicians and measured using a Brass Core Fleisch-type spirometer (Longmont, Colorado, nSpire Health Inc. KoKo PFT Spirometer) with KoKo Moe filters (Longmont, Colorado, nSpire Health Inc.) Accuracy of the KoKo spirometer used is reported by the manufacturer to be ± 1%. Calibration for the spirometer was completed regularly using a 3-Liter syringe according to manufacturer guidelines. Guidelines for spirometry testing were followed according to the

ATS. The same technician performed spirometry before and after work shifts on which dust exposure assessments were also collected. Spirometry technicians were unaware of exposure status. Participant's pulmonary function test efforts were repeated a minimum of three and a maximum of six times before and after shifts. Inability to repeat maximal efforts did not result in exclusion from the study. Spirometry measurements recorded included: forced vital capacity (FVC), FEV_1, the FEV_1/FVC ratio, the forced expiratory flow rate during 25-75% of the FVC (FEF25-75%), and the peak flow expiratory flow rate (PEFR). The best effort of the participants was selected by spirometry software interpretation based on FEV_1. Participant's spirometry tests were compared to the third National Health and Nutrition Examination Survey reference values (Hankinson, Odencrantz, & Fedan, 1999) using gender, age, ethnicity, height weight, and smoking status.

Exhaled Nitric Oxide

An exhaled nitric oxide examination was administered to participants before and after the work shift using the NIOX-MINO (Aerocrine, Morrisville, NC). Techniques followed for measuring eNO followed manufacturer and ATS procedures. Participants inhaled air through a filter connected to the NIOX-MINO in order to remove any non-endogenous nitric oxide. Subjects exhaled slowly into a mouthpiece at a flow rate of 50 ± 5 ml per second for 10 seconds. The NIOX-MINO incorporates graphics and auditory signals to coach the participant to deliver the correct flow rate and duration of exhalation. Concentrations of eNO were recorded by the NIOX-MINO when the correct flow rate and desired duration of exhalation was achieved. Accuracy of the NIOX-MINO is listed by the manufacturer at ± 5 ppb or max 10%, while the range is 5 to 300 ppb.

Statistical Analysis

Descriptive statistics for demographics and characteristics of participants were obtained from questionnaire responses. Exposure concentrations were calculated. Inhalable dust, endotoxin and muramic acid concentrations calculated using the formulas:

*Dust concentration (mg/m^3) = (Mass of dust (mg)/Air sample volume (L)) * (1000 L/ 1 m^3) (Eq. 1)*

Endotoxin units (EU/m^3) = (EU/mg dust Mass dust (mg) / (Air sample volume (L)/1000 L). (Eq. 2)*

*Muramic Acid (ng/m^3) = (ng/mg dust * Mass dust (mg)) / (Air sample volume (L)/1000 L). (Eq. 3)*

Exposure metrics were tested for normality and exposure was found to be lognormally distributed. Cross-shift declines in pulmonary function were calculated using data from spirometry efforts and eNO measurements prior to the shift and immediately after the shift. Cross-shift declines in lung function and measured eNO were distributed normally. Cross-shift lung function values were calculated using the formula:

*Percent change of value = [(Post-shift value – Pre-shift value) / (Pre-shift value * 100)] (Eq. 4)*

Relationships among independent and dependent variables were first tested using Pearson's Product-Moment Correlation Coefficients. Independent variables were considered for multiple regression analysis if correlations with a dependent variable had a p-value equal to or less than 0.2 or if the independent variable was considered a potential confounder. If multiple independent variables met this criterion for a single dependent variable, then independent variables were tested for correlation to each other. If independent variables were correlated with each other at a p-value equal to or less than 0.3, then only one independent variable was chosen for the multiple regression analysis. Cross-shift changes in spirometry measures and eNO concentration were treated as the dependent variables. Independent variables considered for linear regression models included age, ever-smoked, months of farming exposure, months in current job description, hours worked per week, inhalable dust, and inhalable endotoxin. Computations were completed using Minitab (Minitab 16 Statistical Software, 2010. State College, PA Minitab Inc.). To date, relationships between exposure, eNO and Spirometric values have been examined. Additional statistical analyses are being performed to include the remaining 18 repeat study participants.

Results

Of the 62 dairy parlor participants who were enrolled in the study, at least 57 individuals were included in all statistical analyses. Participants were excluded if determined to be outliers or if important information was missing about exposure or health outcomes. A summary of the demographics and characteristics of the 62 study participants is included in Table 2-1. The study population was largely Hispanic males with a few years of farming experience. Years of working on any farm averaged less than five years and a histogram displaying distribution is shown in Figure 2-1. Educational attainment and family income was lower than the United States average. The group's self-reported respiratory symptoms are located within Table 2-2. The geometric means and ranges for inhalable dust and endotoxin and muramic acid exposure concentrations are show in Table 2-3. Study subject self-reported used of personal protective equipment (PPE) for respiratory protection is shown in Table 2-4.

Table 2-1. Characteristics of dairy parlor workers.	Number (%) or mean (SD)
Number of participants	62
Mean age (SD)	31.6 (9.8)
Male	90.3%
Hispanic or Latino	93.5%
Ever-smoked	30.6%
Second hand smoking exposures	42.1%
Used pesticides and/or herbicides in past month	9.6%
Mean months worked in farming	49.8 (46.8)
Mean months in current job description	41.9 (45.2)
Mean hours worked per week	54.4 (12.3)
Currently living on farm	38.7%
Mean years of education	8.1 (3.8)
Median annual family income range	$10,000 - $30,000

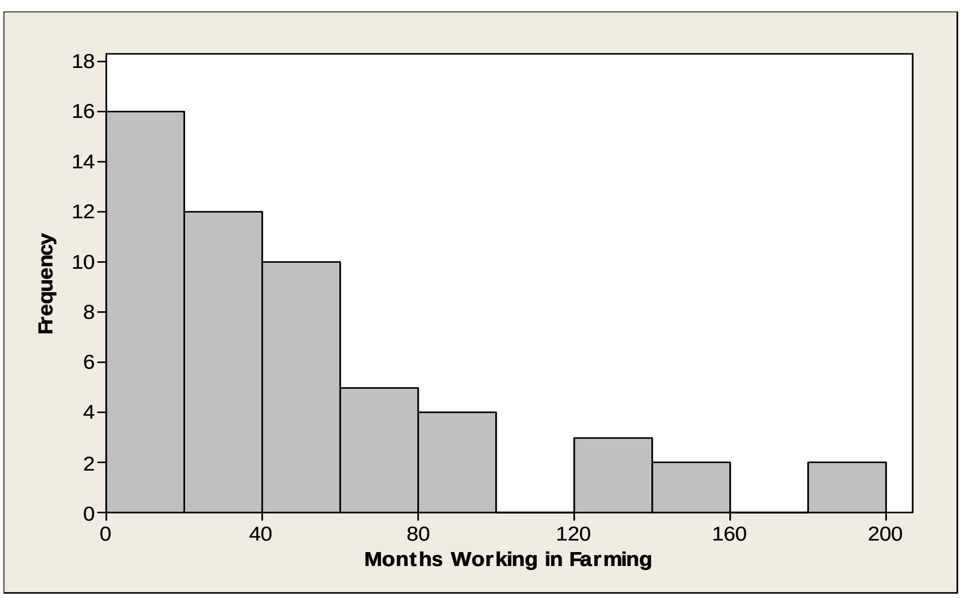

Figure 2-1: Frequency distribution of participant's length of time working in farming.

Table 2-2. Participant self-reported pulmonary symptoms (n=62)

Asthma (physician diagnosed)	4.5%
Usually have cough, phlegm, wheezing, or shortness of breath	16.1%
Usually have cough	7.4%
Usually have phlegm	3.0%
Usually have wheeze	4.7%
Usually have shortness of breath	6.2%
Usually have a headache during work	27.8%
Ever had fever and/or chills after exposure to organic dusts	15.7%
Participants reporting usually having at least one symptom	40.3%

Table 2-3. Participants self-reporting regarding personal perceptions and PPE used during shift sampled. (n=62)

Employee's concerned breathing dust at work or home may cause breathing problems	52%
PPE usage:	
Respirator	0%
Dust mask	5%
Bandana/scarf	5%
None	90%

Table 2-4. Summary measures for occupational exposures of dairy parlor workers.

	n	Geometric Mean (Range)
Inhalable Dust (mg/m^3)	60	0.58 (0.090-5.0)
Inhalable Endotoxin (EU/m^3)	56	118 (4-1968)
Inhalable Muramic acid (ng/m^3)	56	3.59 (0.802-41.0)

Pre-shift spirometry efforts as a percentage of predicted values were calculated for FEV_1, FVC, and FEV_1/FVC. Pre-shift FEV_1 as percentage of predicted values ranged from 72 – 110%, with an average of 93.4%. Pre-shift FVC as a percentage of predicted values ranged from 81 – 137%, with an average of 99.6%. Pre-shift FEV_1/FVC as a percentage of predicted values ranged from 67 – 112%, with an average of 94.2%. Cross-shift changes in FEV_1 were on average decreased by -1.16% with a standard deviation of 4.1%. Cross-shift changes in FVC were on average decreased by -0.01% with a standard deviation of 0.07%. Cross-shift changes in the FEV_1/FVC ratio were increased by 0.01% with a standard deviation of 0.08%. Distributions of exposure metrics compared to measures of health outcome are displayed using scatterplots in Figures 2-2 – 2-10.

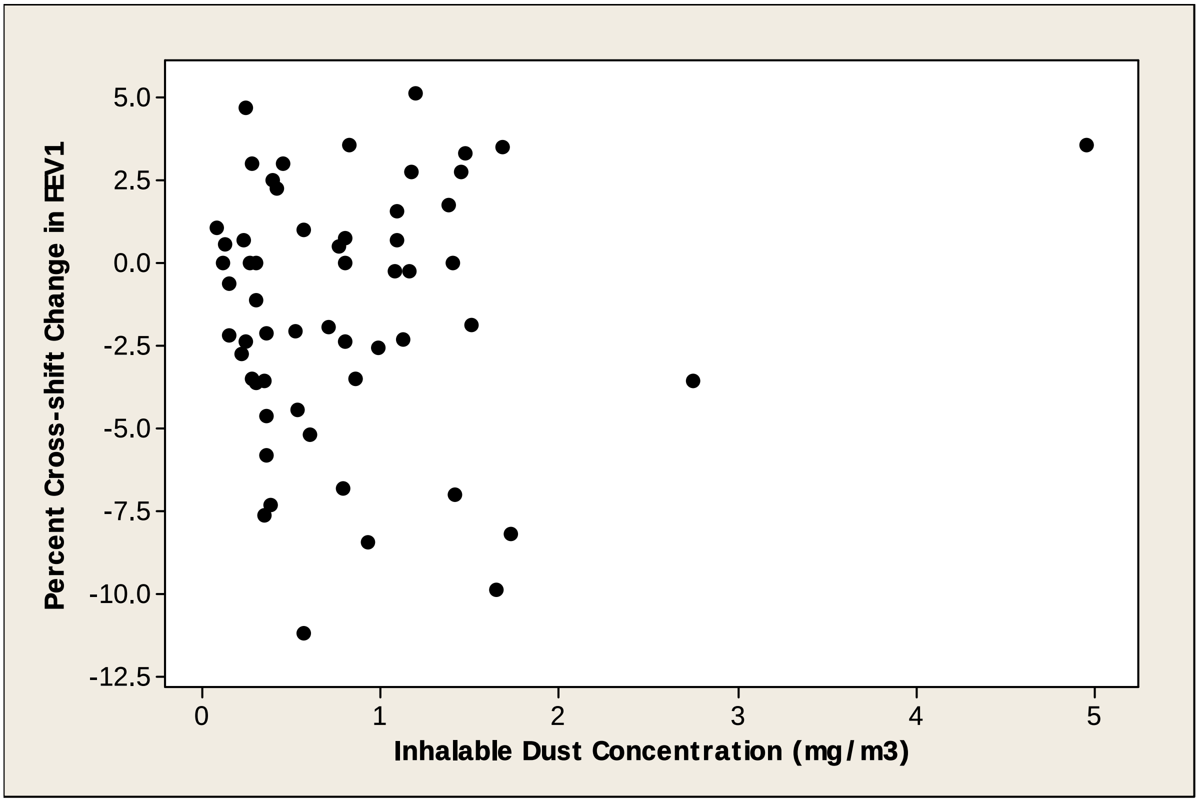

Figure 2-2: Scatterplot of cross-shift changes observed in the FEV_1 and inhalable dust concentrations collected in the personal breathing zone of participants.

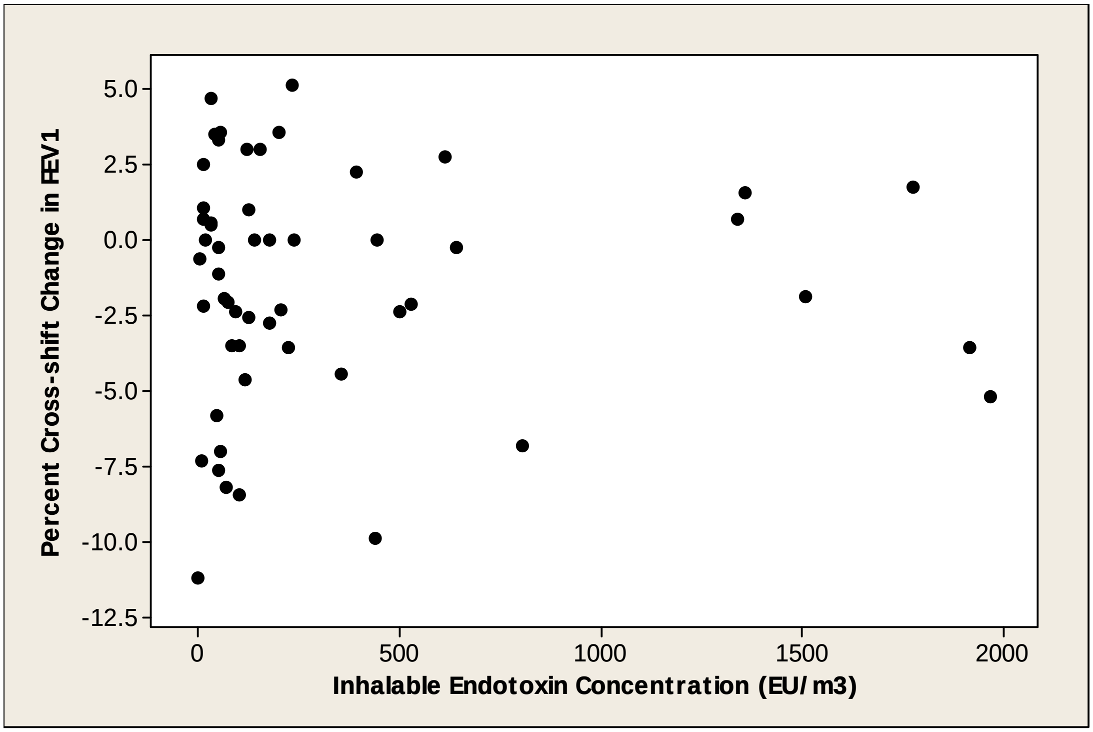

Figure 2-3: Scatterplot of cross-shift changes observed in the FEV_1 and inhalable endotoxin concentrations collected in the personal breathing zone of participants.

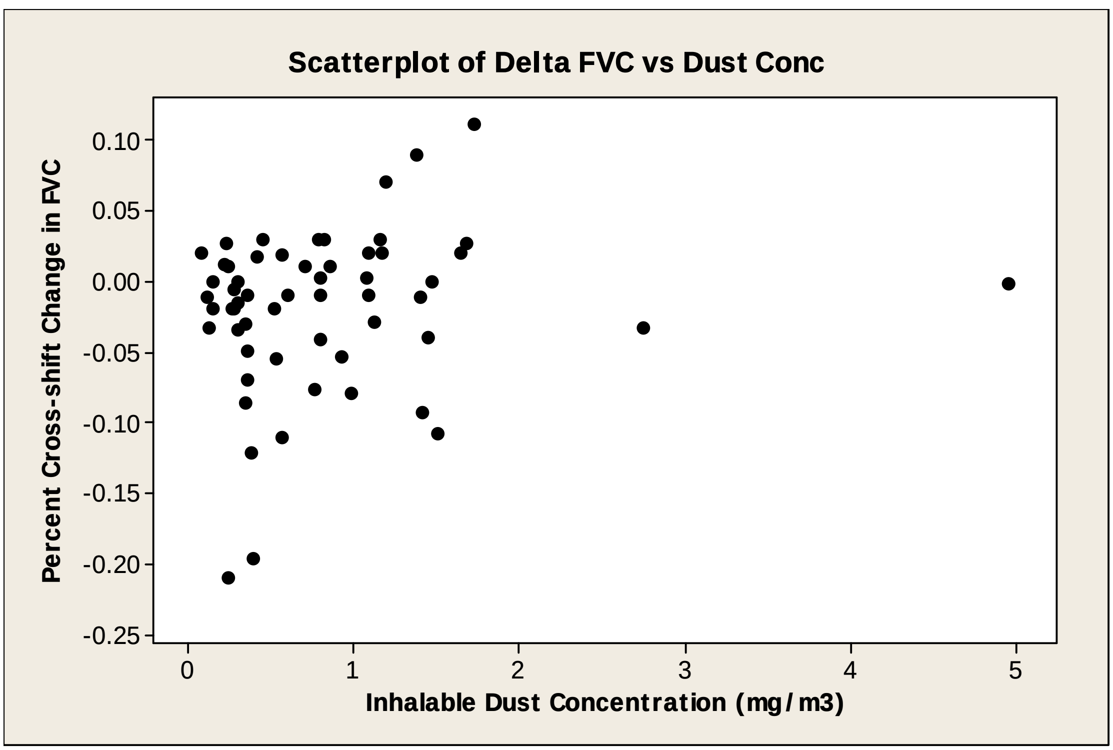

Figure 2-4: Scatterplot of cross-shift changes observed in the FVC and inhalable dust concentrations collected in the personal breathing zone of participants.

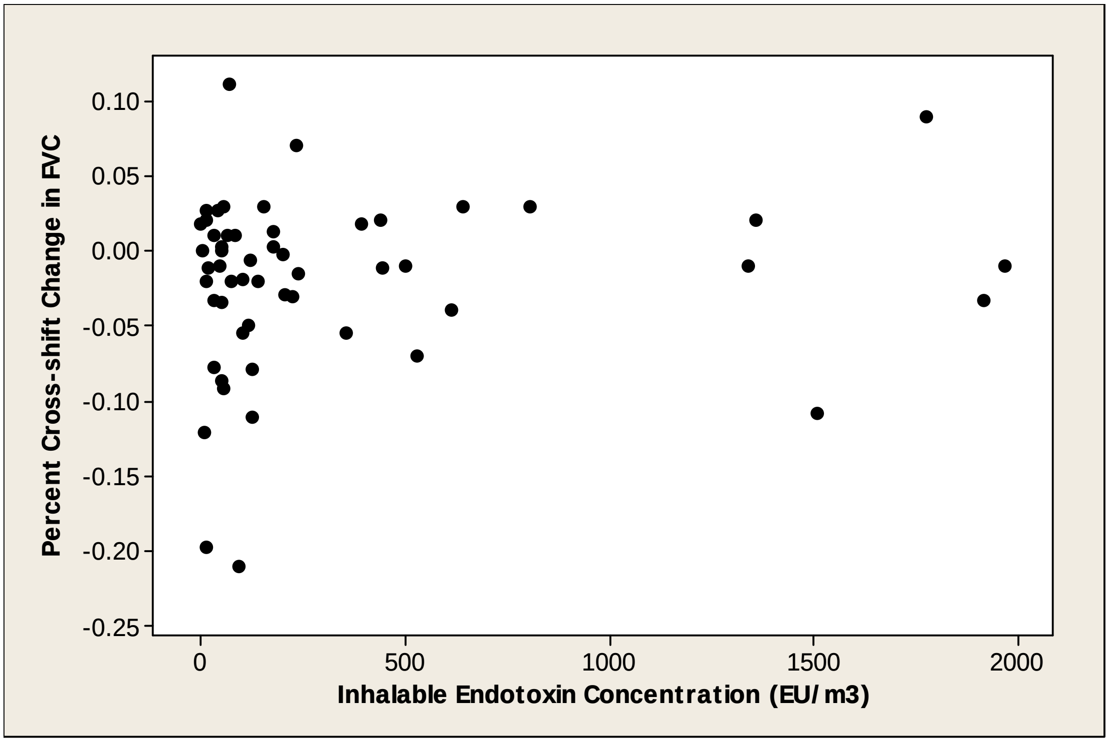

Figure 2-5: Scatterplot of cross-shift changes observed in the FVC and inhalable endotoxin concentrations collected in the personal breathing zone of participants.

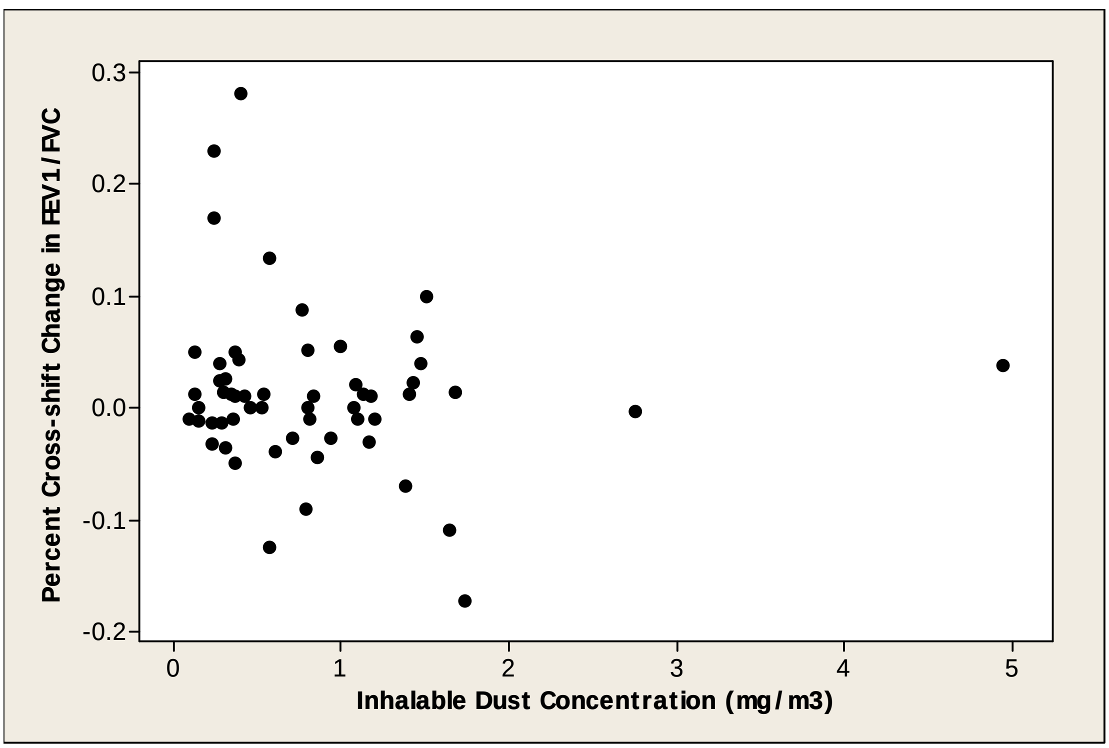

Figure 6: Scatterplot of cross-shift changes observed in the ratio of FEV_1 to the FVC and inhalable dust concentrations collected in the personal breathing zone of participants.

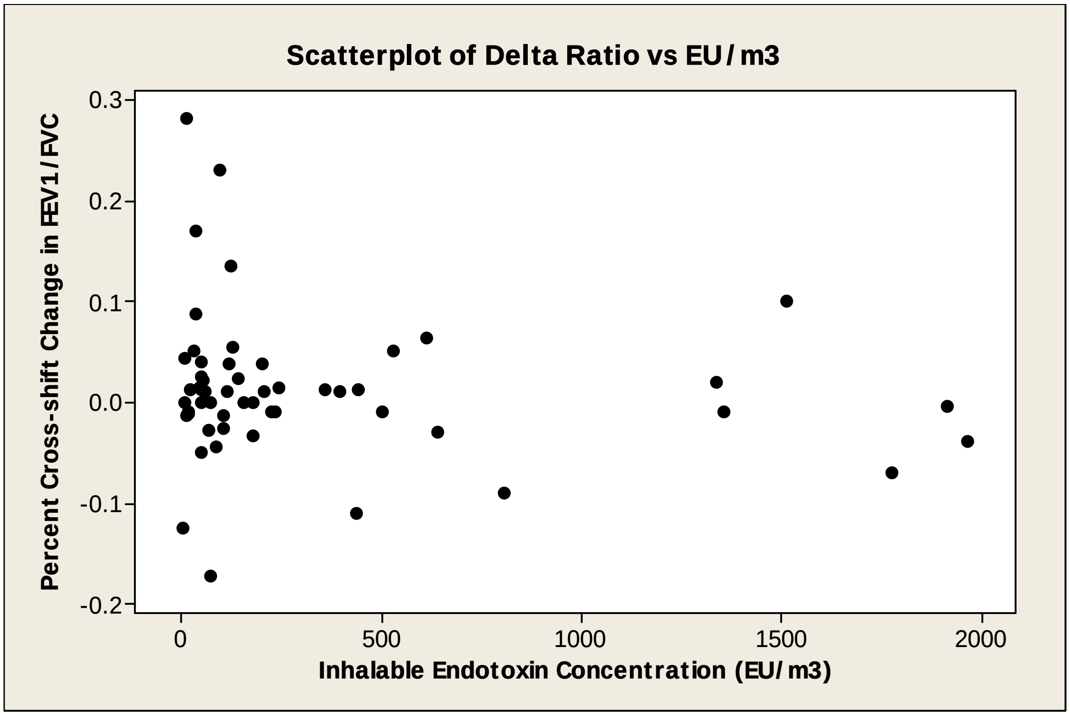

Figure 2-7: Scatterplot of cross-shift changes observed in the ratio of FEV_1 to the FVC and inhalable endotoxin concentrations collected in the personal breathing zone of participants.

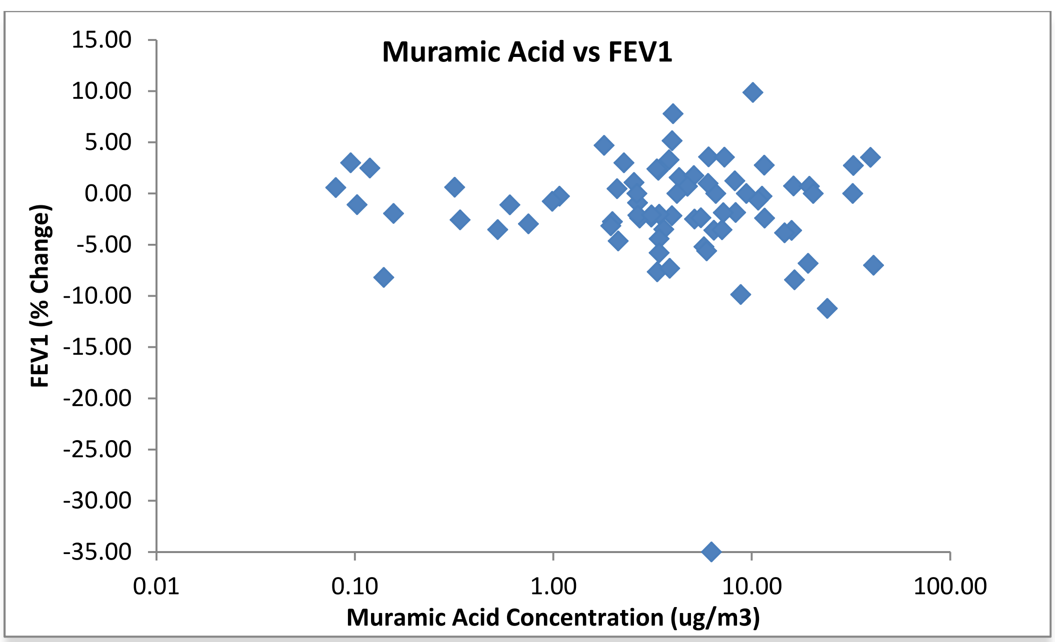

Figure 2-8: Scatterplot of cross-shift changes observed inFEV_1 and inhalable muramic acid concentrations collected in the personal breathing zone of participants.

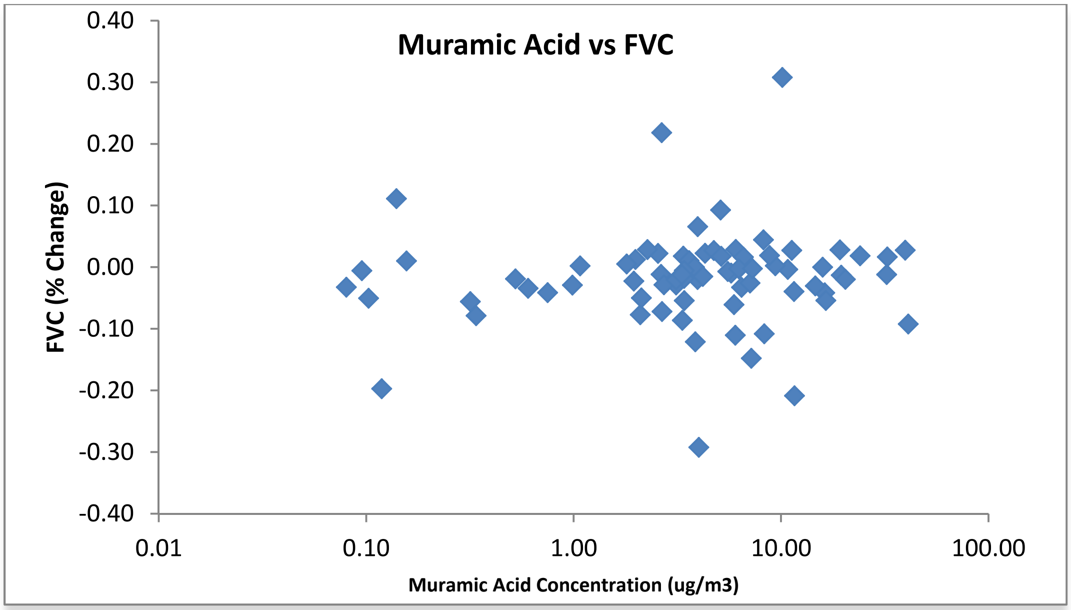

Figure 2-9: Scatterplot of cross-shift changes observed in FVC and inhalable muramic acid concentrations collected in the personal breathing zone of participants.

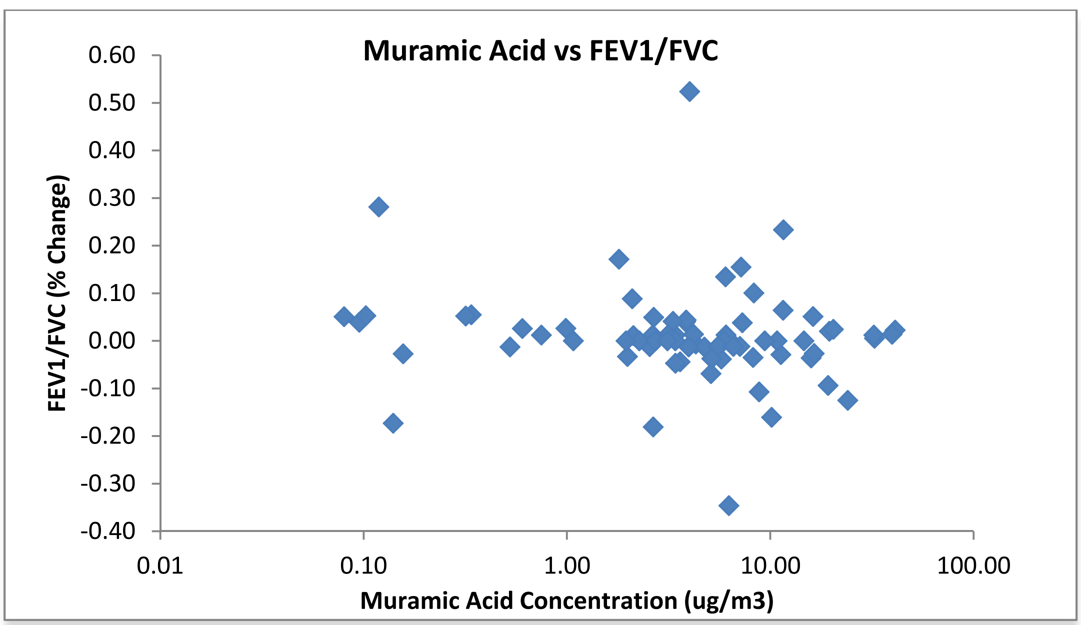

Figure 2-10: Scatterplot of cross-shift changes observed in FEV_1/FVC and inhalable muramic acid concentrations collected in the personal breathing zone of participants.

Cross-shift changes in pulmonary function tests and measurement of exhaled nitric oxide were evaluated for correlations to exposures, work characteristics, and demographic variables. Direct and indirect relationships are described in correlation coefficient matrix shown in Table 2-5. Independent variables were checked for collinearity in Tables 2-6 and 2-7.

The occupational exposure correlation with the strongest inverse coefficient relationships compared to spirometry measures was inhalable endotoxin concentration and percent change in cross-shift changes in the spirometry measure of FEV_1/FVC. The Spearman Correlation Coefficient r-value for inhalable endotoxin and percent change in the cross-shift ratio of FEV_1/FVC was -0.234 with a p-value 0.091. Inverse relationships are expected as increases in organic dust exposures associated with decreases in cross-shift change in pulmonary function measurements have been found in previous studies. Direct relationships were observed between concentrations of inhalable endotoxin and post-shift concentrations of eNO with an r-value of 0.264 and a p-value of 0.052. Tables 2-8 and 2-9 display correlations between eNO measurements, independent variables, and dependent variables. Regression analyses have yet to be performed for muramic acid exposures.

Table 2-5. Pearson Correlation Coefficients between environmental exposure variables and cross-shift changes in measures of bronchial hyperresponsiveness (n=56). P-values listed in bold are less than or equal to 0.2 and were considered for multiple regression analysis.

Variable	% Cross-shift change FEV_1	% Cross-shift change FVC	% Cross-shift change FEV_1/FVC	% Cross-shift change eNO
Inhalable dust				
r values	0.003	0.176	-0.179	0.032
P values	0.981	**0.191**	**0.182**	0.808
Inhalable endotoxin				
r values	-0.050	0.189	-0.234	-0.083
P values	0.724	**0.176**	**0.091**	0.548
Currently living on farm				
r values	0.002	0.141	-0.160	0.088
P values	0.985	0.297	0.236	0.508
Hours worked				

per week				
r values	-0.235	-0.006	-0.163	0.164
P values	**0.079**	0.966	0.225	0.215
Months in job description				
r values	-0.184	-0.072	-0.053	0.014
P values	**0.175**	0.597	0.696	0.914
Months working on farms				
r values	0.013	0.023	-0.032	-0.032
P values	0.929	0.874	0.821	0.817
Ever-smoked				
r value	0.160	0.244	-0.070	-0.310
P values	0.233	**0.068**	0.607	**0.017**
Age				
r values	0.011	-0.043	0.035	0.217
P values	0.933	0.751	0.793	**0.099**
Education				
r values	0.160	0.245	-0.104	-0.028
P values	0.234	**0.067**	0.443	0.833

Table 2-6. Pearson Correlation Coefficients analysis for collinearity between independent variables. P-values listed in bold are less than or equal to 0.3.

Variable	Ever-smoked	Age	Education
Inhalable dust			
r values	-0.059	-0.113	0.175

P values	0.656	0.393	**0.186**
Inhalable endotoxin			
r values	0.213	-0.147	0.308
P values	**0.119**	**0.286**	**0.022**
Currently living on farm			
r values	-0.083	-0.083	0.028
P values	0.533	0.531	0.830
Hours worked per week			
r values	0.035	0.024	-0.032
P values	0.793	0.854	0.810
Months in job description			
r values	**-0.171**	**0.311**	-0.157
P values	**0.200**	**0.022**	**0.240**
Age			
r values	-0.287	1.000	-0.206
P value	**0.028**	0.000	**0.118**
Education			
R values	0.092		1.000
P values	0.489		0.000

Table 2-7. Pearson Correlation Coefficients analysis for collinearity between independent variables. P-values listed in bold are less than or equal to 0.3.

Variable	Inhalable dust	Inhalable endotoxin	Currently living on farm	Hours worked per week	Months in job description
Inhalable dust					
r values	1.000	0.553	-0.034	0.130	0.171
P values	0.000	**0.000**	0.800	0.325	**0.200**
Inhalable endotoxin					
r values		1.000	0.186	-0.056	0.146
P values		0.000	**0.174**	0.683	**0.292**
Currently living on farm					
r values			1.000	-0.287	0.186
P values			0.000	**0.028**	**0.177**
Hours worked per week					
r values				1.000	0.029
P values				0.000	0.828
Months in job description					
r values					1.000
P values					0.000

Table 2-8. Pre and post-shift Pearson Correlation Coefficients for percentage of predicted spirometry and exhaled nitric oxide measurements. P-values listed in bold are less than or equal to 0.2.

Variables	Pre-shift eNO	Post-shift eNO
Inhalable dust		
r values	0.069	0.083
P values	0.603	0.534
Inhalable endotoxin		
r values	0.287	0.264
P values	**0.034**	**0.052**
Months working in farming		
r values	0.152	0.134
P values	0.274	0.333
FEV1 Pre-shift measurement/predicted		
r values	-0.070	-0.157
P values	0.599	0.241

Table2- 9. Characteristics of dairy parlor workers with moderate post-shift eNO values compared to participants with low eNO values. Where appropriate standard deviations are listed in parentheses.

	Moderate* eNO values	Low* eNO values
Number	6	56
Cross-shift change in FEV_1	-3.19%	-0.91%
Mean age	32.3 (9.1)	31.6 (9.9)
Ever-smoked	16.6%	32.1%
Mean months worked in farming	52.2 (71.0)	49.5 (44.2)
Mean months in current job description	52.2 (71.0)	41.0 (42.1)

Mean hours worked per week	54.0 (12.3)	55.6 (9.1)
Currently living on farm	33.3%	44.0%

*Moderate values were defined as eNO measurements between 25 and 50 ppb. Low eNO measurements were defined as less than 25 ppb. The American Thoracic Society's criteria for indication of airway inflammation include: less likely for eNO below 25 ppb and more likely for eNO above 50 ppb. No participants had eNO values above 50 ppb.

Regression equations shown below were developed for: 1) Dependent variable: Cross-shift change in FEV_1, using log-transformed inhalable endotoxin as the independent variable; 2) Dependent variable: Cross-shift % change in FEV_1/FVC, using log-transformed inhalable endotoxin concentration as the independent variable; 3) Dependent variable: Cross-shift change in eNO, using log-transformed inhalable endotoxin as the independent variable; and 4) Dependent variable: Post-shift FEV_1, using pre-shift FEV_1 and log-transformed inhalable endotoxin concentration as the independent variables:

1) Cross-shift % Change FEV_1 = - 0.717- 0.281 log EU/m^3
   - ➢ R-Squared = 0.2%
2) Cross-shift % Change FEV_1/FVC = 0.070 - 0.026 log EU/m^3
   - ➢ R-Squared = 5.5%
3) Cross-shift eNO Change = -0.683 - 0.531 log EU/m^3
   - ➢ R-Squared = 0.7%
4) Post-Shift FEV_1 = – 0.017 log EU/m^3 + 0.997 Pre-shift FEV_1
   - ➢ R-Squared = 94.2%

Multiple regression analysis was performed with the following variables: 1) Dependent variable: Cross-shift % change in FEV_1, using age, hours worked per week and months in current job description as the independent variables; 2) Dependent variable: Cross-shift % change in FEV_1, using age, hours worked per week, months in current job description, and log-transformed inhalable dust concentration as the independent variables; 3) Dependent variable: Cross-shift % change in FEV_1, using age, hours worked per week, months in current job description, and log-transformed inhalable endotoxin concentration as the independent variables; 4) Dependent variable: Cross-shift % change in FVC, using age and log-transformed inhalable dust concentration as the independent variables; 5) Dependent variable: Cross-shift % change in FVC, using age and log-transformed inhalable endotoxin concentration as the independent variables; 6) Dependent variable: Cross-shift % change in FEV_1/FVC, using age and log-transformed inhalable dust concentration as the dependent variables; 7) Dependent variable: Cross-shift % change in FEV_1/FVC, using age and log-transformed inhalable endotoxin concentration as the dependent variables; 8) Dependent variable: Cross-shift change in eNO, using age, ever-smoked, and inhalable dust concentration as the independent variables; and 9) Dependent variable: Cross-shift change in eNO, using age, ever-smoked, and inhalable endotoxin concentration as the independent variables.

1) Cross-shift % change in FEV_1 = [3.43 + 0.0381 Age – 0.0955 Hours per week – 0.0174 Months at current job]

- R-Squared = 9.5%

2) Cross-shift % change in FEV_1 = [3.78 + 0.045 Age – 0.100 Hours per week – 0.019 Months at current job + 0.95 log mg/m^3]

- R-Squared = 10.3%

3) Cross-shift % change in FEV_1 = [1.98 +0.029 Age – 0.059 Hours per week – 0.020 Months at current job – 0.049 log EU/m^3]

- R-Squared = 7.8%

4) Cross-shift % change in FVC = [-0.008 - 0.0002 Age + 0.027 log mg/m^3]

- R-Squared = 3.6%

5) Cross-shift % change in FVC = [0.0545 – 0.0002 Age + 0.017 log EU/m^3]

- R-Squared = 3.2%

6) Cross-shift % change in FEV_1/FVC = [-0.0003 + 0.0002 Age – 0.035 mg/m^3]

- R-Squared = 3.3%

7) Cross-shift % change in FEV_1/FVC = [0.076 – 0.0002 Age – 0.026 EU/m^3]

- R-Squared = 5.5%

8) Cross-shift change in eNO = [-2.97 + 0.069 Age – 2.35 Ever smoked + 0.38 mg/m^3]

- R-Squared = 11.5%
- Dummy variables for ever-smoked: No = 0 Yes = 1

9) Cross-shift change in eNO = [-3.53 + 0.081 Age – Ever smoked -0.036 EU/m^3]

- R-Squared = 11.7%
- Dummy variables for ever-smoked: No = 0 Yes = 1

Section 3

Conclusions

This research project evaluated the exposure of dairy parlor workers to inhalable endotoxin concentrations and cross-shift changes in spirometry and exhaled nitric oxide. The geometric mean endotoxin concentration found in this study's collected samples was less than previously reported by other researchers. This observation may be explained by the cleanliness of the dairy parlor work area resulting in lower exposures compared to other work areas on the dairy farm. The participants in our study performed the majority of their work in the dairy parlor, limiting opportunities for exposures other than those present in the dairy parlor.

Cross-shift changes in lung function using measures of spirometry were noted, including a study group decrease in FEV_1 of 1%. Endotoxin concentrations were not commonly found to be above 1000 EU/m^3, a range previously found to cause acute pulmonary responses (Rylander, 2006). Endotoxin concentrations were found to often be above the 90 EU/m^3 concentration predicted to be the highest exposure concentration without pulmonary responses (Castellan et al., 1987). Cross-shift eNO concentrations did not increase as expected; however participants with higher post-shift eNO measurements were more likely to have decreases in cross-shift FEV_1. Several participants did have low eNO concentrations according to ATS guidelines, while experiencing cross-shift declines in FEV_1. Reasons for decreases in spirometry performance while experiencing no change in eNO are unknown and should be further investigated. It is not known if eNO will have future success in agricultural health screenings, however the ease of use, portability, and simplicity of the NIOX MINO call for future investigations.

Increasing the application of task-based sampling on dairy farms and in the parlor could lead to additional reduction in work exposures in the parlor and else ware on the dairy farm. Agriculture dust controls needs to be effective year-round, economically feasible, and appeal to the farm owner. Dairy farmers are usually aware of cooling/ventilation requirements during the summer when the regulation of animal body temperature is required to maintain optimum milk production. Some research has suggest that parlor fans should be capable of 6 to 12 room air changes per hour. Qualitative assessments during field sampling suggest that ventilation in modern milking facilities is not successful or being used to accomplish 6 to 12 room air changes per hour.

Local exhaust ventilation may be preferred for effective contaminant control; however the return on investment for the cost has yet to be economically evaluated. Incorporating properly engineered local dilution ventilation has potential for effective reduction of dust concentrations and cost savings.

In order to maximize cost efficiency, the number of cows milked in the parlor is maintained at the highest rate possible. In the dairy industry, an empty milking parlor is a milking parlor losing money. Moving cows through the parlor at a rapid pace is fundamental to this philosophy. Using an automatic cow wash pens can decrease prep time in the parlor and result in an increased milking rate (VanBaale, 2004). Washing cows regularly also provides the possibility of reducing the amount of animal dander, feces, and other sources of endotoxin being brought into the parlor.

Considerable weaknesses of these opportunities to reduce parlor endotoxin concentration may include the seasonal feasibility. Ventilation between the milk room and parlor will have effectiveness in the winter while cow washing may be limited to warmer weather. In addition, these suggestions provide potential endotoxin concentration reduction in the parlor while not addressing the stables or corrals. Some dairy personnel in our study reported being trained to use a respirator; however no employees reported respirator usage. Various reasons are likely for the lack of respirator usage; however dairy management should focus on implementing respirator requirements at a minimum for tasks known to generate large amounts of organic dust such as bedding and feeding.

Publications Resulting From This Work

Gallagher M, Nonnenmann MW, Hornick M, Reynolds S, Levin J and Boggaram, V. Measures of Dust, Endotoxin, and Exhaled Nitric Oxide among Dairy Parlor Workers. American Industrial Hygiene Conference and Exposition, Montreal, CA. May 18-23, 2013. Best Student Poster

Hornick M., Nonnenmann MW., Peters T. Evaluating Exposure to Inhalable Dust among Dairy Parlor Workers American Industrial Hygiene Conference and Exposition, Montreal, CA. May 18-23, 2013.

References

American Conference of Governmental Hygienist- Threshold Limit Values for Chemical Substances and Physical Agents (2013). ACGIH, Cincinnati, OH.

Castellan, R. M., Olenchock, S. A., Kinsley, K. B., & Hankinson, J. L. (1987). Inhaled endotoxin and decreased spirometric values. An exposure-response relation for cotton dust. N Engl J Med, 317(10), 605-610. doi: 10.1056/NEJM198709033171005

Dressel, H., Gross, C., de la Motte, D., Sultz, J., Jorres, R. A., & Nowak, D. (2009). Educational intervention in farmers with occupational asthma: long-term effect on exhaled nitric oxide. J Investig Allergol Clin Immunol, 19(1), 49-53.

Donham, K. J., Reynolds, S. J., Whitten, P., Merchant, J. A., Burmeister, L., & Popendorf, W. J. (1995). Respiratory dysfunction in swine production facility workers: dose-response relationships of environmental exposures and pulmonary function. Am J Ind Med, 27(3), 405-418.

Reynolds, S. J., Nakatsu, J., Tillery, M., Keefe, T., Mehaffy, J., Thorne, P. S., . . . O'Shaughnessy, P. (2009). Field and wind tunnel comparison of four aerosol samplers using agricultural dusts. Ann Occup Hyg, 53(6), 585-594. doi: 10.1093/annhyg/mep021

Rylander, R. (2006). Endotoxin and occupational airway disease. Curr Opin Allergy Clin Immunol, 6(1), 62-66. doi: 10.1097/01.all.0000202356.83509.f7

Sparkman, L., Boggaram, Vijayakumar. (2004) Nitric oxide increases IL-8 gene transcription and mRNA stability to enhance IL-8 gene expression in lung epithelial cells. American Journal of Physiology - Lung Cellular and Molecular PhysiologyPublished 1 October 2004Vol. 287no. L764-L773DOI: 10.1152/ajplung.00165.2004ATS/ERS 2005

VanBaale, Matthew. (2004). Managing Personnel for Milking Parlors on Large Herds. MS. Thesis. University of Arizona. http://arizona.openrepository.com/arizona/handle/10150/144744