

Endotoxin Exposure, Inflammation Markers, and Pulmonary Function among Agricultural Workers in Colorado and Nebraska, U.S.A

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Funding: This work was funded by grants from the United States Centers for Disease Control (CDC) National Institute for Occupational Safety and Health (NIOSH): CDC NIOSH R01 OH007841 (New Methods for Evaluation of Organic Dust Aerosols) and CDC NIOSH 5U50 OH008085 (High Plains Intermountain Center for Agricultural Health and Safety).

Abstract

Endotoxin, a constituent of Gram negative bacterial cell walls is an important mediator of adverse respiratory effects from agricultural dust inhalation. This study quantified personal work shift exposures to inhalable dust, endotoxin and its 3-hydroxy fatty acid (3-OHFA) constituents, and evaluated post-work shift nasal lavage fluid inflammation markers and pulmonary function tests before and after the workshift among workers in cattle feedlots, dairies, grain elevators, and corn farms. Geometric mean dust levels (n = 134) were highest among grain elevator operators (4.50 mg/m³) and lowest among farm workers (2.49 mg/m³), whereas geometric mean endotoxin exposure level was highest among feedlot workers (1,093 EU/m³). Livestock dusts contained approximately two times higher concentrations of 3-OHFAs than grain dusts. Patterns of 3-OHFA distribution and proportion of each individual 3-OHFA varied by dust type. Mean PMN, MPO, albumin and ECP levels were 2-3-fold higher among workers in the upper quartile of 3-OHFA exposure compared to the lowest exposure quartile. Even numbered 3-OHFA were most strongly associated with nasal inflammation. 10% of the population with pulmonary function tests (total n = 174) had baseline FEV1 and FEV1/FVC below criteria used to clinically define obstructive lung disease. Close to 50% of the population

had FEV1 and FEV1/FVC less than 95% of predicted – a significant effect for the population from an epidemiological standpoint. Cross shift pulmonary function dropped more than 5% in 26% of the population and more than 10% in 10% of the population. 19% had a drop in FVC exceeding 5% and 8% had an FVC decrease exceeding 10%. The proportions were largest in farmers, followed by dairy workers and grain handlers. Both correlations and regression models indicated that smoking, endotoxin/dust exposure, facility type were significant predictors of symptoms (eye and throat irritation, cough) and pulmonary function (cross shift decrease in FEV1, pre-shift FVC and FEV1). These results suggest that workers with less chronic exposure to work environments with elevated ambient concentrations of endotoxin containing dusts are more susceptible to the acute effects of endotoxin and that extended workplace exposures confer a degree of resistance to the effects of endotoxin, or that sensitive workers who do not adapt leave the industry over time.

Key Words: endotoxin, 3-hydroxy fatty acid, nasal lavage, organic dust, pulmonary function

Introduction

Inhalation of agricultural dusts has been associated with adverse respiratory responses including acute organic dust toxic syndrome (ODTS), chronic asthma and asthma-like syndrome, and chronic obstructive airway disease (Donham, 1995, Reynolds, 1996, Spurzem, 2002 2000, Linaker and Smedley, 2002; Seifert, 2003, Rylander, 2006, Rushton, 2007; Liebers, 2008). Endotoxins are comprised of lipopolysaccharides that are components of Gram negative bacterial cell walls (Mayeux, 1997; Gutschmann et al., 2007; Rylander, 2006; Thorn, 2001). These agents are a common constituent of agricultural dusts and contribute significantly to its pathogenicity. (Rushton, 2007; Rylander, 2006; Seifert et al., 2003; Thorn, 2001). Endotoxin inhalation elicits a potent innate immune response resulting in increased concentrations of cellular and soluble mediators of airway inflammation, as well as increased respiratory symptomatology and changes in quantitative measures of pulmonary function (Linaker and Smedley, 2002; Spurzem et al., 2002; Thorn, 2001).

A relatively high lifetime prevalence of lung disease has been observed among agricultural workers (~6-15% versus ~2-3% in non-farming comparison populations) (Linaker and Smedley, 2002; Spurzem et al., 2002). Some agricultural workers exhibit heightened susceptibility to organic dust's respiratory effects (Castellan et al., 1987;

Dosman et al., 2006; Kline et al., 1999; Schwartz, 2002), resulting in self selection of some workers out of these occupations. Other workers may develop endotoxin tolerance (Broad et al., 2006; Hoffmann et al., 2005; Von Essen and Romberger, 2003), and these factors are thought to contribute, at least in part, to a previously described “healthy worker” effect among agricultural occupations (Bakirci et al., 2006; Post et al., 1998; Rushton, 2007). The mechanisms underlying heightened endotoxin susceptibility or the development of tolerance remain to be determined but likely involve both intrinsic (genetic traits, immune system regulation) and extrinsic factors (work-related behaviors, exposure control processes, dust composition). A better understanding of how these factors contribute to the development of adverse respiratory outcomes is needed to optimize disease prevention efforts.

Few guidelines have been established to prevent the adverse effects of agricultural dust inhalation. A total dust exposure guideline of 2.4 mg/m^3 was recommended to prevent occupational health effects among workers in swine and poultry production facilities (Donham et al., 1995; 2000; 2002; Reynolds et al., 1996). The recommended limits for total endotoxin exposures in these work environments are 614 EU/m^3 and 900 EU/m^3 , respectively (Donham et al., 1995; 2000; Reynolds et al., 1996). These guidelines are based on 37 mm cassette total dust samplers and need to be adjusted for comparison to the IOM (Institute of Medicine) inhalable sampler (Reynolds et al., 2009). The biologically active component of endotoxin, lipid A, is populated by hydroxylated fatty acids of varying carbon chain lengths (Mayeux, 1997; Gutschmann et al., 2007; Larsson, 1994). Endotoxin's 3-hydroxy fatty acid (3-OHFA) moieties can be quantified in agricultural particulate matter via a sensitive and specific gas chromatography / mass spectrometry (GC/MS) method (Pomorska et al., 2007; Reynolds et al., 2005). The acute respiratory effects of endotoxin in other agricultural settings, such as dairies and feedlots, have not been well characterized and no endotoxin specific exposure guidelines have been developed for these work environments. In addition, very few studies have characterized the relationship between 3-OHFA endotoxin constituents and airway responses among workers in any agricultural settings.

In this study, personal exposure to inhalable airborne dust was quantified over one work shift among grain elevator, cattle feedlot, corn farm and dairy workers. Endotoxin in personal airborne dust samples was measured using the recombinant factor C (rFC) assay, as well as a modified GC/MS procedure that quantified endotoxin's 3-OHFA constituents. (Saito 2009) Relationships between each endotoxin exposure measure, post-shift respiratory symptoms, and nasal lavage fluid inflammation markers were examined. (Burch 2009)

Subjects and Methods

Agricultural workers were recruited using different methods for the four groups – cattle feedlots, dairies, grain handling facilities, and corn farmers to accommodate requirements of the producer organizations. The study received institutional review board approval and all participants provided informed consent in either English or Spanish. Data were collected between the spring of 2005 and fall of 2006.

Personal breathing zone samples for inhalable particulate matter were collected using Institute of Medicine (IOM) sampling cassettes and 25 mm PVC filters with a 5 micron pore size (SKC, Eighty Four, PA) at a flow rate of 2 Lpm using personal sampling pumps (MSA, Pittsburgh, PA). Inhalable dust samples were analyzed by weighing the internal cassette and filter as a single unit using a Mettler MT5 balance (Mettler-Toledo, Columbus, OH). Field and lab blanks were analyzed in a similar manner.

Dust samples were extracted in sterile, pyrogen-free water containing 0.05% Tween-20 for 1 hr at room temperature (22°C) with continuous shaking. A portion of each extract was analyzed for endotoxin using the rFC assay (Cambrex, East Rutherford, NJ) as previously described using a microtiter plate reader (Biotek Instruments FLX800TBIE, Winooski, VT) at excitation/emission frequencies of 380/440 nm. (Saito 2009) The detection limit was ~0.01 EU/ml. (Saito 2009) Another portion of the extract was lyophilized and stored at -70 °C for determination of 3-OHFA endotoxin constituents via a GC/MS method modified for these environments (Saito 2009). Samples and standards derivatized with N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) were analyzed using an HP 5890 Series II Plus gas chromatograph equipped with an HP-5MS column (Hewlett-Packard, Palo Alto, CA), and an HP 5972 Mass Selective Detector. Selected ion monitoring was used for individual 3-OHFA, and results were quantified in picomoles (pmols). Calibration was accomplished via lab fortified matrix blanks at anticipated 3-OHFA concentrations in dust samples. The limit of detection (LOD) and the limit of quantitation (LOQ) were determined by signal-to-noise (S/N) ratio based on the chromatograms of controls and 0.5 and 1 ng spikes (S/N >3 for LOD and >10 for LOQ) (1980).

Self-administered, structured, written questionnaires were used to gather information on respiratory health, mucous membrane irritation symptoms, tobacco smoke exposure and workplace exposures before and after a work shift. These questionnaires were constructed using the American Thoracic Society standardized questionnaire (Ferris 1978). Additional questions for identifying ODTs were based on Rylander (1990).

A nasal lavage fluid sample was collected at the end of each participant's work shift using a previously described protocol with 5 ml of normal (0.9%) saline instilled into each nostril (Naclerio et al., 1983). Cell pellets and frozen nasal lavage fluid supernatants were shipped via express mail to the National Institute for Occupational Safety & Health (NIOSH) Bio-Organic Chemistry Laboratory where they were analyzed using commercially available immunoassay kits as described previously. (Burch 2009) The following inflammation marker proteins were quantified: myeloperoxidase (MPO) (Assay Designs, Inc., Ann Arbor, MI); Eosinophil Cation Protein (ECP) (Phadia, Portage, MI, formerly Pharmacia CAP System™, Pharmacia Diagnostics, Uppsala, Sweden); albumin (Bethyl Laboratories, Montgomery, TX); and Interleukin-8 (IL-8) (R&D Systems, Minneapolis, MN) (Gaughan et al., 2008; Woodin et al., 1998). The limits of quantification were 500 pg/mL, 630 pg/mL, 1.6 pg/mL and 6.25 ng/mL for ECP, MPO, IL-8 and albumin, respectively.

Spirometry was performed in triplicate with the subjects in a seated position using the Puritan Bennett Renaissance spirometer (Puritan Bennett; Wilmington, MA). Forced Vital Capacity (FVC), Forced Expiratory Volume in 1 second (FEV₁), and their ratio were measured. The best value was reported according to American Thoracic Society standards. Spirometry was performed before the work shift began and again at the end of the work period. Interpretation of the spirometry findings was done using prediction equations from Hankinson and colleagues (Hankinson 1999) and the interpretation algorithm of the American Thoracic Society and European Respiratory Society taskforce for the standardization of lung function testing (Pellegrino 2005). The FEV₁/FVC value was defined as demonstrating airway obstruction if it was less than the 5th percentile of predicted. The FVC value was defined as being consistent with but not diagnostic of restriction if it was less than the 5th percentile of predicted.

Statistical analyses were performed using the SAS computer program (version 9.1, SAS Institute Inc., Cary, NC). Exposure and inflammation marker variables were analyzed using log-transformed data. Associations among exposures, inflammation markers, and pulmonary function were evaluated by calculating Spearman rank correlation coefficients (r). The Proc GLM procedure was used to evaluate differences among groups. Stepwise multiple linear regression (inclusion/exclusion p = 0.05) was used to evaluate the relationships between pulmonary function, endotoxin/dust exposure, and smoking.

Results and Discussion

A total of 174 participants from 26 work sites were recruited into the study. Characteristics of the study population are presented in Table I. On average participants were 37 years of age, had been at their current job for 8.5 years and had worked in agriculture for 16 years. Grain elevator workers and farmers were older, with more experience. 28% of the population was Hispanic or Latino. 25% were current smokers, although none of the farmers smoked.

TABLE I. Characteristics of Study Population

| Characteristic | Grain Elevator (n=76) | | Feedlot (n=71) | | Dairy (n=18) | | Farm (n=9) | | Total (n=174) | |
|---|-----------------------|----------|----------------|----------|--------------|----------|------------|----------|---------------|----------|
| | Ave | Stdev | Ave | Stdev | Ave | Stdev | Ave | Stdev | Ave | Stdev |
| Age (years) | 41 | 13 | 34 | 12 | 30 | 9 | 38 | 13 | 37 | 13 |
| Time at Current Job (years) | 9.2 | 11.0 | 7.8 | 9.3 | 4.8 | 5.9 | 14.4 | 11.0 | 8.5 | 10.0 |
| Hours worked this week | 18.6 | 17.4 | 32.3 | 20.8 | 25.0 | 17.1 | 22.4 | 20.7 | 25.0 | 19.9 |
| Working in area with Visible Dust (hours/day) | 8.3 | 10.9 | 10.8 | 15.9 | 29.6 | 27.1 | 19.1 | 22.8 | 11.9 | 16.8 |
| | <i>n</i> | <i>%</i> | <i>n</i> | <i>%</i> | <i>n</i> | <i>%</i> | <i>n</i> | <i>%</i> | <i>n</i> | <i>%</i> |
| Ethnicity | | | | | | | | | | |
| Hispanic or Latino | 12 | 16 | 17 | 24 | 16 | 89 | 3 | 33 | 48 | 28 |
| Not Hispanic or Latino | 59 | 78 | 50 | 70 | 1 | 6 | 9 | 100 | 116 | 67 |
| Refuse/don't know/missing | 5 | 7 | 4 | 6 | 1 | 6 | 0 | 0 | 10 | 6 |
| Education | | | | | | | | | | |
| Elementary | 2 | 3 | 11 | 15 | 5 | 28 | 1 | 11 | 19 | 11 |
| High School | 52 | 68 | 24 | 34 | 11 | 61 | 2 | 22 | 89 | 51 |
| College | 21 | 28 | 36 | 51 | 1 | 6 | 5 | 56 | 63 | 36 |
| Refuse/don't know/missing | 1 | 1 | 0 | 0 | 1 | 6 | 1 | 11 | 3 | 2 |
| Ever smoked cigarettes | | | | | | | | | | |
| 31 | 41 | 21 | 30 | 9 | 50 | 3 | 33 | 64 | 37 | |
| Current Smokers: | | | | | | | | | | |
| Heavy (>47 packs/year) | 20 | 26 | 15 | 21 | 4 | 22 | 0 | 0 | 39 | 22 |
| Low (<47 packs/year) | 2 | 3 | 1 | 1 | 2 | 11 | 0 | 0 | 5 | 3 |

Figure 1 shows the geometric mean (GM) and geometric standard deviation (GSD) of dust, endotoxin, and 3-OHFA levels for the four agricultural environments. A significant difference was found for dust concentration ($p = 0.03$) and endotoxin concentration per mg dust ($p < 0.01$) among the agricultural environments, specifically between cattle feedlots and grain elevators ($p < 0.05$). Grain elevators had the highest geometric mean

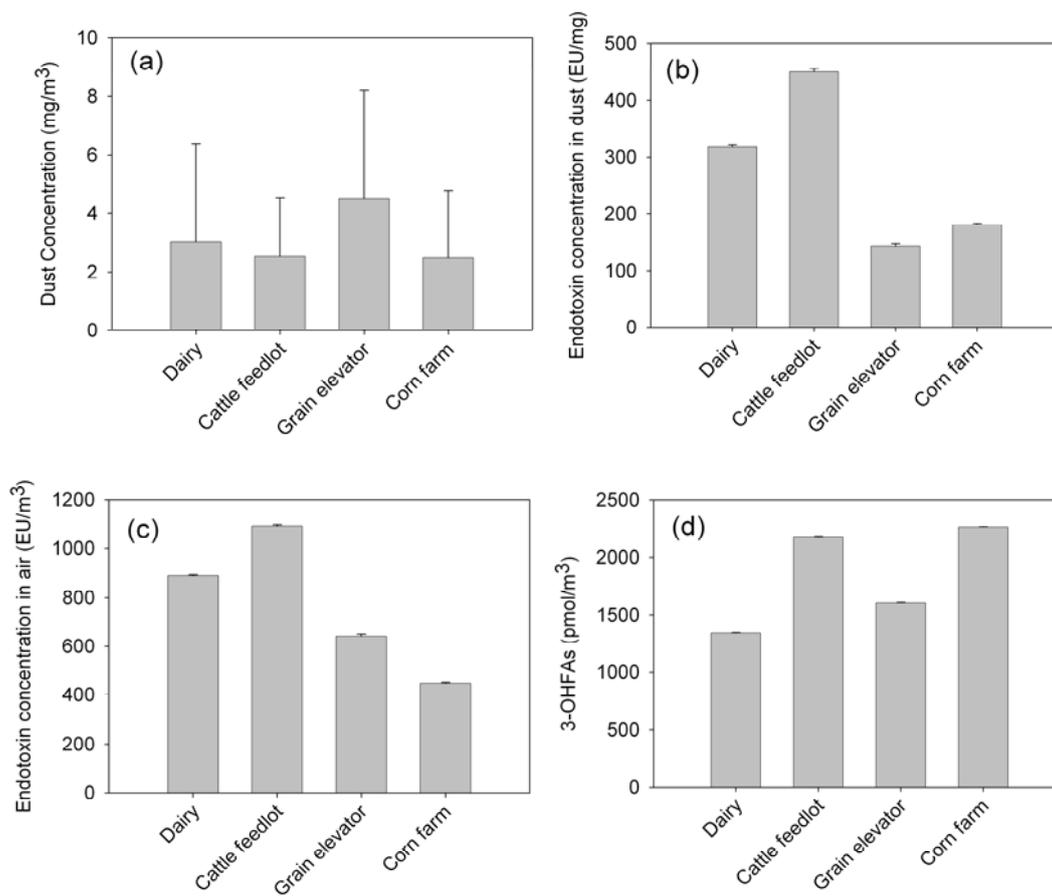


FIGURE 1. GM with error bar (GSD) of (a) dust concentration, (b) endotoxin concentration per mg dust, (c) endotoxin concentration per m³ air, and (d) GC/EI-MS endotoxin result (total 3-OHFAs) per m³ air.

dust concentration (4.50 mg/m³), followed by dairies (3.02 mg/m³), cattle feedlots (2.54 mg/m³), and corn farms (2.49 mg/m³). Endotoxin concentrations were the highest among cattle feedlot workers (450 EU/mg and 1,093 EU/m³). Total 3-OHFA (sum of 3-OHFAs) level per m³ air was the highest in corn farms (2,266 pmol/m³) and the lowest among grain elevator operators (1,345 pmol/m³), although the difference among agricultural environments for total 3-OHFA levels was not statistically significant (p = 0.31). Figure 2 shows the differences in the patterns of individual 3-OHFAs by environment. C12 and C14 dominate in all four environments. 125 men had complete data available for nasal lavage/immune marker analysis. Detailed results are reported in Burch 2009. Mean MPO

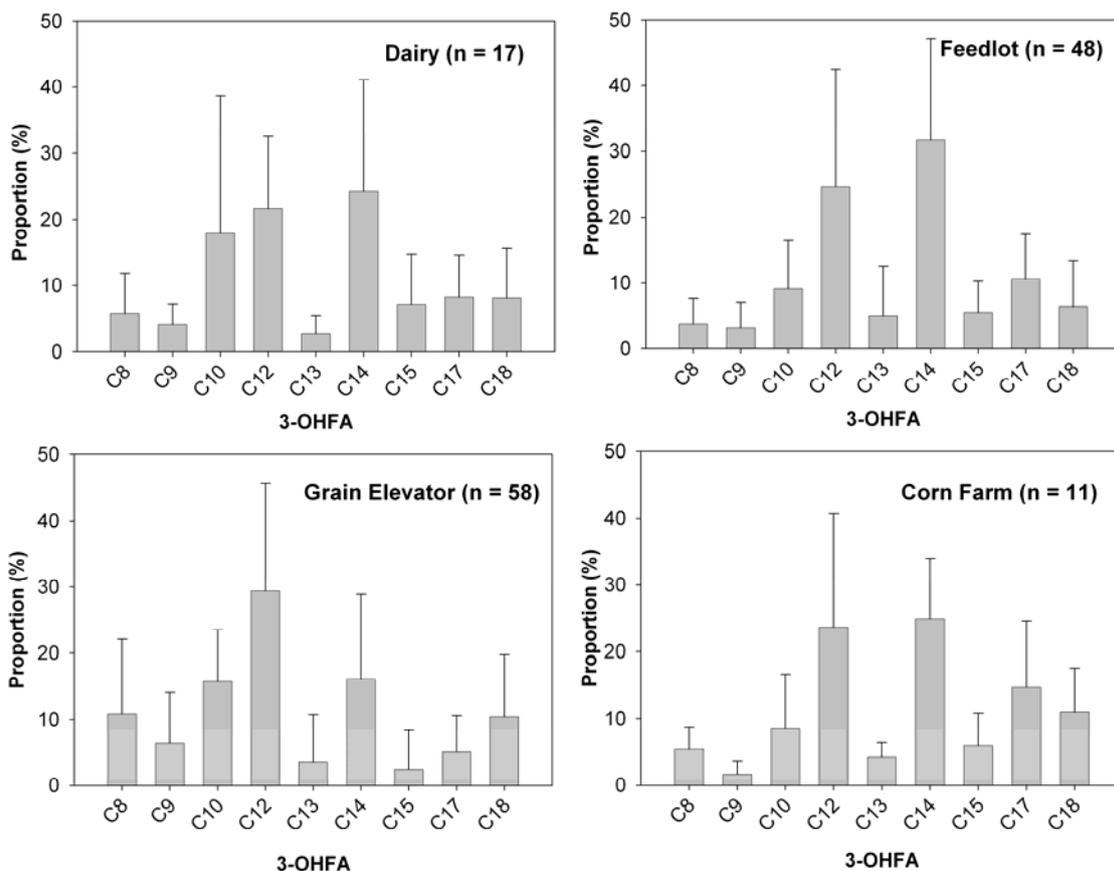


Fig 2. Mean relative proportion of 3-OHFA with standard deviation (error bar) found in each dust type by GC/EI-MS analysis.

levels were greater among Caucasians and non-Hispanics and rose with increasing age, job duration, or duration of farm work. Nasal lavage fluid IL-8 concentrations were elevated among: workers in cattle feedlots, dairies and corn farms compared to those working in grain elevators; workers with a longer duration of farm work; and those located at work sites where livestock was kept (242 vs. 153 pg/mL). An increased frequency or duration of work in grain elevators or corn storage areas was associated with a reduction in IL-8 concentrations. A similar pattern of reductions in mean albumin levels was observed among workers with an increased frequency but not duration of grain elevator or corn storage work. ECP concentrations tended to rise with longer job duration or with a greater frequency or duration of farm work. Mean nasal lavage PMN counts (1,672 vs. 476 cells/ml), ECP (2.55 vs. 0.97 ng/ml), or albumin concentrations (12,922 vs. 4,978 ng/ml) were elevated among those reporting post-shift headaches compared to those without headaches. Mean albumin

levels were also higher among those with excessive phlegm following their work shift (7,352 vs. 4,711 pg/mL). Measurable increases in mean lavage fluid ECP concentrations were noted among participants with post-shift cough (1.45 vs. 0.96 pg/mL). Endotoxin exposures were associated with statistically significant increases in IL-8 concentrations. When endotoxin exposures were characterized using the sum of the 3-OHFA (pmol/mg), there were statistically significant elevation in PMN, MPO, and ECP with increasing 3-OHFA exposure. Some nasal lavage inflammation markers were also elevated in response to total 3-OHFA normalized to the air volume sampled (pmol/m³), although only changes in mean MPO attained statistical significance. The most robust increases in lavage fluid inflammation markers were observed to be directly related to exposure to even numbered carbon chain length 3-OHFA.

Table II . Pulmonary Function - % Predicted, Overall and by Facility Type Mean (SD)

| PFT Parameter | All N = 174 | Grain N = 76 | Feedlot N = 71 | Dairy N = 18 | Farm N = 9 | ANOVA R² | ANOVA P = |
|---|--------------------|---------------------|-----------------------|---------------------|-------------------|----------------------------|------------------|
| Pre-FVC | 97.7 (12.1) | 96.7 (12.9) | 97.1 (11.4) | 98.4 (12.3) | 109 (11.3) | 0.19 | 0.02 |
| Pre-FEV₁ | 95.9 (13.6) | 94.0 (13.2) | 96.1 (13.6) | 95.9 (11.5) | 109.7 (15.4) | 0.21 | <0.01 |
| Pre-FEV₁/FVC | 98.5 (7.0) | 97.7 (8.6) | 99.0 (7.0) | 98.7 (7.5) | 101.5 (10.8) | 0.10 | 0.51 |
| %Cross shift decrease in FVC | 1.0 (7.5) | 1.4 (8.6) | 0.3 (5.4) | - 0.6 (9.8) | 6.8 (5.8) | 0.14 | 0.16 |
| %Cross shift decrease in FEV₁ | 1.8 (8.6) | 2.1 (10.2) | 1.0 (7.0) | 1.3 (7.8) | 6.0 (5.8) | 0.10 | 0.57 |
| %Cross shift decrease in ratio | 0.6 (5.3) | 0.8 (5.7) | 0.5 (5.5) | 0.5 (4.6) | - 0.3 (0.8) | 0.09 | 0.64 |

ANOVAs accounting for smoking, hours worked, years worked, mg/m³

Table II. presents baseline and cross shift pulmonary function results. Farmers had significantly higher baseline FVC, and FEV1. Cross shift FVC and FEV1 decreased much more for farmers (6.8% and 6.0%), but the difference was not statistically significant, possibly due to the small numbers (n = 9). Cross shift FEV1 decreased 1.0 to 2.1% for the other groups. 10% of the population had baseline FEV1 and FEV1/FVC below criteria (Gold II, Celli 2003) used to clinically define obstructive lung disease

(Table III),(we did not do post bronchodilator testing which would be used for clinical confirmation).

TABLE III. Baseline Pulmonary Function below Criteria for Obstruction

| | Grain Elevator (n=76) | | Feedlot (n=71) | | Dairy (n=18) | | Farm (n=9) | | Total (n=174) | |
|------------------------------------|-----------------------|----|----------------|----|--------------|----|------------|----|---------------|----|
| | n | % | n | % | n | % | n | % | n | % |
| FEV₁ <80% | 9 | 12 | 7 | 10 | 1 | 6 | 1 | 11 | 18 | 10 |
| FEV₁/FVC <88% | 10 | 13 | 5 | 7 | 1 | 6 | 1 | 11 | 17 | 10 |
| FEV₁ <95% | 42 | 55 | 33 | 46 | 8 | 44 | 1 | 11 | 84 | 48 |
| FEV₁/FVC <95% | 24 | 32 | 16 | 23 | 3 | 17 | 2 | 22 | 45 | 26 |

The rates were lowest among dairy workers and highest among grain workers. Close to 50% of the population had FEV₁ and FEV₁/FVC less than 95% of predicted – a significant effect for the population from an epidemiological standpoint (Pelligrino 2005). Again the rates were highest for grain elevator workers. Cross shift pulmonary function changes exceeding a drop of 5% and 10% are shown in Table IV. Overall FEV₁ dropped more than 5% in 26% of the population and more than 10% in 10% of the population. 19% had a drop in FVC exceeding 5% and 8% had and FVC decrease exceeding 10%. Normally FEV₁ and FVC would be expected to increase by 5% over a daytime workshift. The reductions in pulmonary function were largest in farmers (who also had the highest 3 OHFA exposures), followed by dairy workers and grain handlers.

TABLE IV. Cross Shift Change in Pulmonary Function exceeding 5% and 10%

| | Grain Elevator (n=76) | | Feedlot (n=71) | | Dairy (n=18) | | Farm (n=9) | | Total (n=174) | |
|---|-----------------------|----|----------------|----|--------------|----|------------|----|---------------|----|
| | n | % | n | % | n | % | n | % | n | % |
| FEV₁ positive change | 36 | 48 | 26 | 37 | 7 | 39 | 2 | 22 | 71 | 41 |
| FEV₁ >5% decrease | 20 | 27 | 13 | 18 | 6 | 33 | 6 | 67 | 45 | 26 |
| FEV₁ >10% decrease | 8 | 11 | 5 | 7 | 2 | 11 | 2 | 22 | 17 | 10 |
| FVC positive change | 30 | 40 | 32 | 45 | 6 | 33 | 1 | 11 | 69 | 40 |
| FVC >5% decrease | 13 | 17 | 10 | 14 | 4 | 22 | 6 | 67 | 33 | 19 |
| FVC >10% decrease | 7 | 9 | 3 | 4 | 1 | 6 | 3 | 33 | 14 | 8 |
| FEV₁/FVC positive change | 31 | 41 | 26 | 37 | 8 | 44 | 6 | 67 | 71 | 41 |
| FEV₁/FVC >5% decrease | 8 | 11 | 5 | 7 | 2 | 11 | 0 | 0 | 15 | 9 |
| FEV₁/FVC >10% decrease | 2 | 3 | 2 | 3 | 0 | 0 | 0 | 0 | 4 | 2 |

The most commonly reported pre-shift symptoms were mucus, cough, and irritation of throat, nose, and eyes. The overall rates for these lower and upper respiratory symptoms were comparable to previous studies of agricultural workers and higher than expected in a non-exposed population (e.g. NHANES III). There were no significant increases in rates of symptoms reported by facility type except for cough, mucus, and tingling fingers. Rates of symptoms in almost all cases were lowest in dairy workers and highest in farmers. Cross shift symptoms decreased at similar or greater rates than they increased. Symptoms with the largest cross shift increase included eye and nose irritation, and mucus.

Exposures were not correlated with symptoms except for smoking and endotoxin exposure. Smoking was positively correlated with pre-shift eye and throat irritation and cough, negatively associated with cross-shift throat irritation and cough. Endotoxin exposure was correlated with pre- shift blurred vision and post-shift throat irritation. Log endotoxin and log dust exposures were also correlated with cross shift change in FEV1. Log pmol/m^3 and individual log 3-OHFAs (primarily even numbered carbon chains) were correlated with both baseline and cross-shift decrease in FVC, FEV1, and their ratio. Log $\text{pmol} \times \text{hours}$ and log $\text{pmol} \times \text{years}$ correlated with cross shift decrease in FVC, FEV1, and their ratio. Smoking, especially average daily number of cigarettes, was correlated with baseline FVC and FEV1. Type of facility (which also represents dust type) was correlated with baseline FEV1 and with baseline shortness of breath.

Multiple regression models for predictors of pulmonary function were not strong (low R^2) for both baseline and cross shift PFTs. Variables tested included age, type of facility, hours of work, years of work, smoking, log mg/m^3 , log EU/mg or log EU/ m^3 or log pmol/m^3 (dust and endotoxin were highly correlated and not used in the same models). Exposure \times hours of work and exposure by years of work were also evaluated. Including dust or endotoxin exposures and smoking into the models did not appreciably improve them. Dropping farmers also did not consistently or appreciably improve models. The best models, based on R^2 and Mallows Cp, included average daily number of cigarettes, log $\text{pmol} \times \text{years}$ for baseline FVC, FEV1, ratio, and age, time at job (hours per week), and pre-PFTs for cross shift change in FVC, FEV1, ratio.

Logistic regression models retained age in models predicting baseline shortness of breath, cross shift change in shortness of breath, and change in nose irritation. Hours of work the previous week was retained in models predicting baseline cough, baseline throat irritation, and cross shift change in cough. All odds ratios were very close to 1.0, but were significant.

It is possible that the weaker associations found in this study were partially due to the “health worker effect” wherein the most sensitive workers leave the industry with those remaining exhibiting resistance to the effects of endotoxin. It is also possible that 3-OHFAs from sources in addition to Gram negative bacteria (e.g. fungi, plant material), may also contribute to variability or error and thus reduce the strength of associations. Additional detail on variation in exposures in different job tasks, and seasons were beyond the scope of this paper and will be addressed in additional publications.

Conclusions

In summary, this study quantified personal work shift exposures to inhalable dust, endotoxin, and its 3-OHFA constituents among workers in several agricultural settings. Exposures were compared to biomarkers of inflammation in post-work shift nasal lavage fluids. The cross-sectional nature of this study did not allow for causal associations between 3-OHFA exposures and adverse respiratory outcomes to be evaluated. Nonetheless, elevated concentrations of 3-OHFA in workplace ambient particulate matter were associated with robust, statistically significant increases in nasal airway inflammation, indicating that these agents may serve as important indicators of the biological potency of airborne agricultural dust. This was the first study to evaluate the relationship between endotoxin's 3-OHFA constituents in agricultural dust and nasal airway inflammation. A significant proportion of the population (10%) had baseline FEV1 and FEV1/FVC below criteria (Gold II, Celli 2003) used to clinically define obstructive lung disease. Cross shift pulmonary function also dropped more than 5% in 26% of the population and more than 10% in 10% of the population. 19% had a drop in FVC exceeding 5% and 8% had and FVC decrease exceeding 10%. The proportions were largest in farmers (who had the highest 3 OHFA exposures), followed by dairy workers and grain handlers. Both correlations and regression models indicate that smoking, endotoxin or 3OHFA exposure, and facility type were significant predictors of symptoms (eye and throat irritation, cough) and pulmonary function (cross shift decrease in FEV1, pre-shift FVC and FEV1). These results suggest that workers with less chronic exposure to work environments with elevated ambient concentrations of endotoxin containing dusts are more susceptible to the acute effects of endotoxin and that extended workplace exposures confer a degree of resistance to the effects of endotoxin, or that sensitive workers who do not adapt leave the industry over time.

Acknowledgements

The authors thank the Colorado Corn Growers Association, Pinnacol Assurance, the Colorado Livestock Association, and the Colorado Grain and Feed Handlers Association for assistance with the identification of eligible workers. The authors thank Jason Nakatsu Colene Andersen, and Opal Christensen for assistance with data collection, Dr. Donald Milton for consultation on the GC/MS method, and Debjani Chanda and Mike Wirth for assistance with data analyses. Cambrex provided support for the rFC assay kits. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

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