ENDOTOXIN EXPOSURE AND RESPIRATORY OUTCOMES AMONG DAIRY, FEEDLOT, AND GRAIN ELEVATOR WORKERS IN COLORADO

Scope of Problem

More than 1,000,000 men, women, and children are at risk for occupational lung disease related to organic dust exposure in the U.S.

Components of organic dust include fecal matter, animal skin, feathers, feed and bedding components, insects, fungi, bacteria and microbial products.
Clinical and Epidemiological Studies

Wilkins et al: cash grain farmers, chronic cough 9%, phlegm 11%, dysphnea 16%, wheeze 8%

Schenker et al 1998 – asthma, ODTS, chronic bronchitis

NIOSH: fatalities from hypersensitivity pneumonitis
Clinical and Epidemiological Studies

- Reynolds et al: exposure and respiratory symptoms in turkey barn workers –

- Reynolds, Donham, Thorne, Merchant, et al: occup. asthma 20%, chronic bronchitis 25%, ODTS 33% (Swine, Poultry, Dairy)

- Suggested OELs for Swine, Poultry environments
  - Dust 2.5 mg/m³,
  - Endotoxin 1,000 EU/m³,
  - Ammonia 7 ppm)
Endotoxins

- Lipid A portion – Pathogen Associated Molecular Pattern (germ line encoded receptors)
- Role in immune system modulation and asthma –
  - Adaptation or down-regulation of response

- Genetic risk factors (CD14, TLR4)
  - LeVan and Von Essen (2005) CD14 and PFT decrements
Objectives

1) Characterize worker exposure to endotoxin-containing corn dust aerosols;

2) Evaluate respiratory outcomes including symptoms, cross shift changes in pulmonary function, (PFT) and cellular/immune markers (cytokines);

3) Survey genetic markers related to lung disease and endotoxin etiology (TLR4 gene mutations, and polymorphisms of IL1-RN, and TNF-alpha);

4) Explore whether endotoxin assay or GC/MS is best predictor of biomarkers, PFTs, Sx;

5) Explore whether cellular/immune responses and PFT differ among those with different genetic status.
Research Team

Colorado State University
Stephen Reynolds, PhD, CIH
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University of Nebraska
Susanna Von Essen

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Nell Burch, Ph.D.

NIOSH
Paul Siegel, Ph.D.

PI (Exposure/Epi)
Epidemiology
Biostatistics
Analytical Chemistry
Industrial Hygiene
Occ Medicine/Epi
IH/Chemistry
Occ. Medicine
Pulmonary Medicine
Genetics
Biomarkers
Population

- N = 250 Workers, > 18 years
- Corn Growers Association
- Grain Handlers Association
- Colorado Livestock Association
- Nebraska – Grain Handlers and Farmers

ATTENTION!
COLORADO CORN GROWERS & GRAIN STORAGE FACILITY OWNERS

A STUDY OF LUNG FUNCTION AMONG CORN WORKERS 18 YEARS OR OLDER will be conducted by the Colorado State University High Plains InterMountain Center of Agricultural Health and Safety (PHAMS) in Northern and Eastern Colorado to find out how exposed are corn workers to a component of dust known as endotoxins. Our reason is to develop methods for preventing disease in agricultural workers and their families.

WHAT'S INVOLVED?
A CSU team would visit your farm or storage facility and conduct tests about dust. Each person would participate over one work day. The CSU team will ask workers to:

1. Complete a questionnaire about work history and health status.
2. Wear an air sampling pump on their belt over one work shift.
3. Blow into the tube of a lung function test machine.
4. Provide a blood sample and nasal swab.

The lung function test will be repeated at the end of the work shift.

WHAT'S IN IT FOR YOU?
Each participant will receive a written report of their breathing test results and a summary of the overall study results.

Cash, Gift Card: A CSU baseball cap or $25 in appreciation for each participant's contribution.

ALL DATA AND INFORMATION WILL BE CONFIDENTIAL TO REGULATORY AGENCIES AND INVOLVED IN THE STUDY.

INTERESTED? NEED MORE DETAILS?
CSU will be sending out a letter asking farm and storage facility owners to learn more and sign up for the study. Those interested will be contacted by the researcher. To register, call PHAMS at 970-491-4152.

YOUR PARTICIPATION CAN MAKE A DIFFERENCE!!!!!!!!!!
Methods

Pre- Work Shift
- Exposure/Respiratory Health Questionnaire
  - Based on ATS and Organic Dust (Rylander, Donham)
- Pulmonary Function Test (Spirometrics 2500)
- Blood Sample – Genetics (TLR4 gene mutations Puregene)

Exposure Measurement
- IOM Personal Dust Sample

Post – Work Shift
- Respiratory Questionnaire
- Pulmonary Function Test
- Nasal Lavage
  - (Cytokines IL-4, IL-8, TNF-α, ECP, myeloperoxidase)
Organic Dust/Endotoxin Sampling

- IOM Personal Sampler
- Gravimetric Analysis
- rFC Endotoxin Assay
- GC/MS Endotoxin Analysis

Figure 1. Schematic drawing of the endotoxin detection mechanisms in the LAL system and the rFC system.
Demographics

- N = 81
- Mean age = 33 yrs (19 – 57)
- 98.8% Male
- 51.9% Hispanic/Latino
- 53% White
- 35.8% Other
- 1.2% Asian
- 3.7% American Indian
Characteristics

**Smoking**
- 26% cigarettes
- 1% cigars
- 8% chewing tobacco
- 3.5% snuff

**Respiratory Protection**
- 10.6% used dust mask
- 1.2% used respirator with cartridge
## Exposure Results - Overall

**N = 81**

<table>
<thead>
<tr>
<th></th>
<th>Mg/m³</th>
<th>EU/mg</th>
<th>EU/m³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geometric Mean</td>
<td>3.13</td>
<td>393</td>
<td>1,288</td>
</tr>
<tr>
<td>GSD</td>
<td>2.8</td>
<td>4.2</td>
<td>5.8</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.36</td>
<td>34.7</td>
<td>29.5</td>
</tr>
<tr>
<td>Maximum</td>
<td>75.9</td>
<td>13,500</td>
<td>50,100</td>
</tr>
</tbody>
</table>
Personal Dust Exposures By Facility Geometric Means (ANOVA p = 0.017)

- Farms (n=7) Mg/m^3
- Grain (n=22) Mg/m^3
- Feedlots (n=34) Mg/m^3
- Dairy (n=17) Mg/m^3
Personal Endotoxin Exposures By Facility Geometric Means (ANOVA p = 0.001 EU/mg)
## Exposure Results – Overall Pearson Correlations

**N = 81**

<table>
<thead>
<tr>
<th></th>
<th>Mg/m³</th>
<th>EU/mg</th>
<th>EU/m³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg/m³</td>
<td>1.0</td>
<td>NS</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>P &lt;0.01</strong></td>
</tr>
<tr>
<td>EU/mg</td>
<td></td>
<td>1.0</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>P &lt;0.01</strong></td>
</tr>
<tr>
<td>EU/m³</td>
<td></td>
<td></td>
<td>1.0</td>
</tr>
</tbody>
</table>
Multiple Linear Regressions
GC/MS 3-OHFA with rFC

<table>
<thead>
<tr>
<th>Combinations of 3-OH FA</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>OVERALL</td>
<td>14, 17</td>
</tr>
<tr>
<td>Grain elevator</td>
<td>13, 14</td>
</tr>
<tr>
<td>Feedlot</td>
<td>13, 14, 18</td>
</tr>
<tr>
<td>Dairy</td>
<td>18</td>
</tr>
</tbody>
</table>

with 90% significance level
Cross – Shift Change in Pulmonary Function Results
Overall N = 81
Mean(SD)

<table>
<thead>
<tr>
<th>FEV1%</th>
<th>FVC%</th>
<th>FEV1/FVC%</th>
</tr>
</thead>
<tbody>
<tr>
<td>-3.1 (5.6)</td>
<td>-2.0 (6.0)</td>
<td>-1.4 (3.0)</td>
</tr>
</tbody>
</table>
## Cross – Shift Change in Pulmonary Function Results

### Mean (SD)

<table>
<thead>
<tr>
<th></th>
<th>FEV1%</th>
<th>FVC%</th>
<th>FEV1/FVC%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farms N = 7</td>
<td>-8.1 (2.9)</td>
<td>-9.1 (3.2)</td>
<td>0.97 (2.0)</td>
</tr>
<tr>
<td>Grain N = 22</td>
<td>-3.0 (6.6)</td>
<td>-1.6 (6.0)</td>
<td>-2.4 (4.1)</td>
</tr>
<tr>
<td>Feedlots N = 34</td>
<td>-1.8 (5.0)</td>
<td>-0.6 (5.8)</td>
<td>-1.3 (2.1)</td>
</tr>
<tr>
<td>Dairy N = 17</td>
<td>-4.2 (5.3)</td>
<td>-2.8 (5.7)</td>
<td>-1.3 (3.2)</td>
</tr>
</tbody>
</table>

**ANOVA**

- \( P = 0.039 \)
- \( P = 0.006 \)
- \( P = 0.088 \)
Pearson Correlations PFTs

**N = 81**

<table>
<thead>
<tr>
<th></th>
<th>FEV1</th>
<th>FVC</th>
<th>FEV1/ FVC</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV1</td>
<td>1.0</td>
<td>0.82</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P &lt; 0.1</td>
</tr>
<tr>
<td>FVC</td>
<td></td>
<td>1.0</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P = 0.02</td>
</tr>
<tr>
<td>FEV1/ FVC</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Prevalence of Symptoms (n = 81)

\{ANOVA – shortbreath > grain p = 0.015\}
Immune Markers
{ANOVA – IL8 > farmers (1099) p = 0.014,
ECP > farmers (17.9 p = 0.039)
Pearson Correlations (all p < 0.05)

<table>
<thead>
<tr>
<th>Immune</th>
<th>r</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL8</td>
<td>0.74</td>
<td>18</td>
</tr>
<tr>
<td>IL4</td>
<td>0.54</td>
<td>18</td>
</tr>
<tr>
<td>IL4</td>
<td>0.54</td>
<td>18</td>
</tr>
<tr>
<td>IL4 Log</td>
<td>0.54</td>
<td>18</td>
</tr>
<tr>
<td>ECP dFVC</td>
<td>-0.30</td>
<td>56</td>
</tr>
</tbody>
</table>
Pearson Correlations (all p < 0.05)

<table>
<thead>
<tr>
<th>Pre Symptoms</th>
<th>r</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough</td>
<td>0.48</td>
<td>18</td>
</tr>
<tr>
<td>EU/m3</td>
<td>-0.25</td>
<td>80</td>
</tr>
<tr>
<td>Log mg/m3</td>
<td>-0.22</td>
<td>79</td>
</tr>
<tr>
<td>EU/mg</td>
<td>-0.31</td>
<td>80</td>
</tr>
<tr>
<td>EU/m3</td>
<td>-0.37</td>
<td>81</td>
</tr>
</tbody>
</table>
Pearson Correlations (all $p < 0.05$)

<table>
<thead>
<tr>
<th>Pre Symptoms</th>
<th>r</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short breath</td>
<td>Log EU/mg</td>
<td>-0.23</td>
</tr>
<tr>
<td>Eye irrit.</td>
<td>IL4</td>
<td>0.50</td>
</tr>
<tr>
<td>Nose irrit.</td>
<td>TNF$\alpha$</td>
<td>0.56</td>
</tr>
</tbody>
</table>
Pearson Correlations (all $p < 0.05$)

<table>
<thead>
<tr>
<th>Post Symptoms</th>
<th>r</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucus</td>
<td>0.22</td>
<td>80</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nose irrit.</td>
<td>0.48</td>
<td>18</td>
</tr>
<tr>
<td>TNFα</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short breath</td>
<td>0.46</td>
<td>55</td>
</tr>
<tr>
<td>TLR4-299</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short breath</td>
<td>0.40</td>
<td>55</td>
</tr>
<tr>
<td>TLR4-399</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Pearson Correlations (all \( p < 0.05 \))

<table>
<thead>
<tr>
<th>PFT</th>
<th>r</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>dFVC</td>
<td>IL4</td>
<td>0.54</td>
</tr>
<tr>
<td>dFVC</td>
<td>ECP</td>
<td>0.30</td>
</tr>
<tr>
<td>dFEV1</td>
<td>ECP</td>
<td>0.30</td>
</tr>
<tr>
<td>dFEV1/FVC</td>
<td>MPO</td>
<td>-0.29</td>
</tr>
<tr>
<td>dFEV1/FVC</td>
<td>Log mg/m3</td>
<td>0.40</td>
</tr>
</tbody>
</table>
Regression (stepwise)

- **FEV1/FVC**
  - logmg/m3, \( R^2 = 0.183 \)

- **Post Cough**
  - smoking, \( R^2 = 0.091 \)

- **Post Short Breath**
  - TLR4 299, log EU/m\(^3\), \( R^2 = 0.269 \)

- **Post Mucus**
  - smoking, \( R^2 = 0.82 \)

- **IL4**
  - Log mg/m3, \( R^2 = 0.29 \)
Interpretation

- Personal exposures to dust and endotoxin quite variable.
- Some endotoxin and dust levels very high.
- Means exceed current recommended OELs
- Dust (grain > farm > feedlot > dairy)
- Endotoxin (feedlot > farm > dairy > grain)
Interpretation

Respiratory Outcomes—

- Irritation Sx increased over shift, resp Sx decreased
- FEV1 (flow) and FVC (capacity) decrease
- Change in FEV1 (farm>>dairy>grain>feedlot)
- Change in FVC (farm>>dairy>grain>feedlots)
Interpretation

- Cross-shift change in FEV1/FVC and IL4 associated with log mg/m³.
- Pre-shift cough, wheeze associated with dust and endotoxin levels.
- Post-shift cough and mucus associated with smoking.
- TLR4 299 and endotoxin associated with shortness of breath.
Future

- Data collection
- Genetic markers
- Cytokines
- GC-MS
- Naïve Dairy Workers Study
- CA Dairy Worker Study
- Collaboration with IAREH comparing populations
Acknowledgements

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