Endotoxin Exposure, Immune Markers, and Pulmonary Function in Agricultural Workers in Colorado and Nebraska

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Background: Clinical and Epidemiological Studies


- 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/m$^3$,
- Endotoxin 1,000 EU/m$^3$,
- Ammonia 7 ppm)
Endotoxins

- Lipopolysaccharide-protein complexes
- Potency varies among G- bacteria species
- Cell activation (neutrophils, macrophages)
- Mediator release (interleukins)
- Complement activation
- Decrements in PFTs, ODTS, HP
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 agricultural 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.
Recruitment

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

- Pre-Work Shift
  - Exposure/Respiratory Health Questionnaire
    - Based on ATS and Organic Dust (Rylander, Donham)
  - Pulmonary Function Test
    - (Spirometrics 2500, Puritan Bennett Renaissance, NHANES III)
  - Blood Sample – Genetics (TLR4 gene mutations, and polymorphisms of IL1-RN, and TNF-alpha)

- Exposure Measurement
  - IOM Personal Dust Sample
    - rFC Assay
    - GC/MS

- Post-Work Shift
  - Respiratory Questionnaire
  - Pulmonary Function Test
  - Nasal Lavage (Cytokines)
Organic Dust/Endotoxin Sampling

- IOM Personal Sampler
- Gravimetric Analysis
- rFC Endotoxin Assay
- GC/EI-MS Endotoxin Analysis (HP 5890 Series II Plus GC, HP5972 Mass Selective Detector)

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

<table>
<thead>
<tr>
<th></th>
<th>Grain Elevator N = 76</th>
<th>Cattle Feedlot N = 71</th>
<th>Dairy N = 18</th>
<th>Farm, Corn N = 9</th>
<th>Total N = 174</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>41</td>
<td>34</td>
<td>30</td>
<td>38</td>
<td>37</td>
</tr>
<tr>
<td>Years Work</td>
<td>12.7</td>
<td>20.4</td>
<td>9.9</td>
<td>23.4</td>
<td>16.1</td>
</tr>
<tr>
<td>Hispanic/Latino</td>
<td>16%</td>
<td>24%</td>
<td>89%</td>
<td>33%</td>
<td>28%</td>
</tr>
<tr>
<td>Current Smokers</td>
<td>29%</td>
<td>22%</td>
<td>33%</td>
<td>0%</td>
<td>25%</td>
</tr>
</tbody>
</table>

- **98.8% Male**
Frequency of Tasks by Operation

- Truck harvest
- Combine harvest
- Loading/unloading
- Running legs (in elevator)
- Housekeeping/cleaning
- Mechanical maintenance
- Feeding livestock

<table>
<thead>
<tr>
<th>Task</th>
<th>Dairy</th>
<th>Feedlot</th>
<th>Grain Elevator</th>
<th>Farm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency of response (%)</td>
<td>80</td>
<td>60</td>
<td>40</td>
<td>20</td>
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</table>
Inhalable Dust by Operation

Dairy
Cattle feedlot
Grain elevator
Farm

Dust concentration (mg/m$^3$)

0.1
1
10
100

Dairy
Cattle feedlot
Grain elevator
Farm
Inhalable Endotoxin by Operation

Endotoxin concentration (EU/m$^3$)

<table>
<thead>
<tr>
<th>Operation</th>
<th>1</th>
<th>0</th>
<th>0</th>
<th>0</th>
<th>0</th>
<th>0</th>
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</thead>
<tbody>
<tr>
<td>Dairy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cattle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grain Elevator</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farm</td>
<td></td>
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</table>
Inhalable Dust Exposure by Task

<table>
<thead>
<tr>
<th>Task</th>
<th>Dust concentration (mg/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage</td>
<td>0.1</td>
</tr>
<tr>
<td>Sampling</td>
<td>1</td>
</tr>
<tr>
<td>Milling</td>
<td>10</td>
</tr>
<tr>
<td>Bagging feed</td>
<td>100</td>
</tr>
<tr>
<td>Feeding livestock</td>
<td>1000</td>
</tr>
<tr>
<td>Supervising</td>
<td>1000</td>
</tr>
<tr>
<td>Other</td>
<td>1000</td>
</tr>
</tbody>
</table>
Inhalable Endotoxin by Task

[Bar chart showing distribution of inhalable endotoxin concentration by task, with categories including Storage, Mixing, Milling, Feeding livestock, Weighing, Sampling, Harvesting, and other. The chart includes a log scale on the y-axis and task categories on the x-axis.]
Exposures –
Geometric means (geometric standard deviation)
Risk Factors for High Exposures
(Multiple Linear Regression)

- **Dust**
  - Grain elevator operator (+), hours working in feed storage (+), hours running legs in grain elevator (+), and hours supervising (-).

- **Endotoxin**
  - Grain elevator operator (-), farm worker (-), hours in feed storage (+), hours running legs in grain elevator (+), hours supervising (-), and years in the job (-).
Variability in 3 OHFA by Dust Type

Dairy (n = 17)

Feedlot (n = 48)

Grain Elevator (n = 58)

Farm (n = 11)
## Correlations: GC/MS and rFC Assay

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>R</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>Overall</td>
<td>134</td>
<td>0.4306</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Dairy</td>
<td>17</td>
<td>0.5332</td>
<td>0.0275</td>
</tr>
<tr>
<td>Cattle Feedlot</td>
<td>48</td>
<td>0.7155</td>
<td>&lt; 0.0001</td>
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<tr>
<td>Grain Elevator</td>
<td>58</td>
<td>0.1145</td>
<td>0.3922</td>
</tr>
<tr>
<td>Corn Farm</td>
<td>11</td>
<td>0.3288</td>
<td>0.3235</td>
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</table>
# Multiple Regressions: rFC Assay and 3-OHFAs

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>3-OHFA Combination</th>
<th>R²</th>
</tr>
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<tbody>
<tr>
<td>Overall</td>
<td>134</td>
<td>14, 8, 10</td>
<td>0.3314</td>
</tr>
<tr>
<td>Dairy</td>
<td>17</td>
<td>17, 10</td>
<td>0.6527</td>
</tr>
<tr>
<td>Cattle Feedlot</td>
<td>48</td>
<td>14, 18, 13</td>
<td>0.7329</td>
</tr>
<tr>
<td>Grain Elevator</td>
<td>58</td>
<td>9, 10, 17</td>
<td>0.2480</td>
</tr>
<tr>
<td>Corn Farm</td>
<td>11</td>
<td>None</td>
<td>-</td>
</tr>
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</table>
## Correlations:
### Odd and Even Length 3-OHFAs

<table>
<thead>
<tr>
<th>Location</th>
<th>Endotoxin activity vs. 3-OHFAs</th>
<th>Even vs. Odd 3-OHFAs</th>
</tr>
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<tbody>
<tr>
<td>Dairy</td>
<td>Even</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>Odd</td>
<td>0.60</td>
</tr>
<tr>
<td>Cattle Feedlot</td>
<td>Even</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>Odd</td>
<td>0.70</td>
</tr>
<tr>
<td>Grain Elevator</td>
<td>Even</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>Odd</td>
<td>-0.24</td>
</tr>
<tr>
<td>Corn Farm</td>
<td>Even</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>Odd</td>
<td>0.15</td>
</tr>
</tbody>
</table>
Mean Inflammation Marker Levels (N=125)

† - p-value for characteristic as a continuous variable in parentheses. a, b - p≤0.05, p≤0.01 vs. former tobacco users, respectively. c - p≤0.05 vs. current cigarette / cigar smoker. PMN - polymorphonuclear neutrophils. MPO - myeloperoxidase. IL interleukin. ECP - eosinophilic cation protein.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>PMN (cells/ml)</th>
<th>MPO (ng/ml)</th>
<th>IL-8 (pg/ml)</th>
<th>Albumin (ng/ml)</th>
<th>ECP (ng/ml)</th>
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<tbody>
<tr>
<td><strong>Age Group</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>18-24 years</td>
<td>(0.62)†</td>
<td>(0.05)</td>
<td>(0.19)</td>
<td>(0.39)</td>
<td>(0.25)</td>
</tr>
<tr>
<td>(n=37)</td>
<td>446</td>
<td>30</td>
<td>245</td>
<td>6,634</td>
<td>0.99</td>
</tr>
<tr>
<td>25-40 years</td>
<td>545</td>
<td>40</td>
<td>181</td>
<td>4,447</td>
<td>1.07</td>
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<tr>
<td>(n=57)</td>
<td>665</td>
<td>55</td>
<td>200</td>
<td>6,003</td>
<td>1.16</td>
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<tr>
<td>41-72 years</td>
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<td></td>
<td></td>
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<tr>
<td>(n=31)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>Type of Facility</strong></td>
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<tr>
<td>GrainElevator</td>
<td>438</td>
<td>33</td>
<td>154</td>
<td>5,110</td>
<td>0.93</td>
</tr>
<tr>
<td>(n=46)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Feedlot</td>
<td>528</td>
<td>50</td>
<td>221&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5,715</td>
<td>1.11</td>
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<tr>
<td>(n=55)</td>
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</tr>
<tr>
<td>Dairy</td>
<td>744</td>
<td>41</td>
<td>245&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5,385</td>
<td>1.07</td>
</tr>
<tr>
<td>(n=15)</td>
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<tr>
<td>Farm</td>
<td>992</td>
<td>32</td>
<td>390&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6,048</td>
<td>1.69</td>
</tr>
<tr>
<td>(n=9)</td>
<td></td>
<td></td>
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</tbody>
</table>
Summary of Immune Marker Results

- Associations with the 3-OHFAs were greater than those observed with endotoxin or inhalable dust exposure.
- Mean PMN, MPO, albumin and ECP levels were two- to three-fold higher among workers in the upper quartile of 3-OHFA exposure compared to the lowest exposure quartile.
- Even chain length 3-OHFAs were most strongly associated with nasal inflammation.
Symptoms with Increase Over Workshift

- Eye Irritation (31%)
- Nose Irritation (99%)
Baseline PFT below Criteria for Obstructive Lung Disease (Gold II, Celli 2003)

- FEV1<80%
- FEV1<95%
- FEV1/FVC<88%
- FEV1/FVC<95%
Mean Cross Shift Change in PFT
(5% increase is normal)
Proportion with Cross Shift Decrease in PFT Exceeding 5% and 10%
Predictors of Baseline PFT
(Correlations and Multiple Linear Regression)

- FVC – Log Endotoxin (EU/m³, pmol/m³) concentration, type of facility, smoking.

- FEV1 – Log Endotoxin concentration (EU/m³, pmol/m³) type of facility, smoking.

- FEV1/FCV – Log Endotoxin (EU/m³, pmol/m³) or Log Dust concentration, type of facility, smoking.

- Correlations ($r = 0.15 – 0.24$) and regressions ($R^2 0.02 – 0.12$) weak in all cases

- C12, C14, C18 3OHFAs best models for FVC, FEV1, FEV1/FVC.
Predictors of Cross Shift Decline in PFT

(Correlations and Multiple Linear Regression)

- FVC – Log Endotoxin (EU/m$^3$, pmol/m$^3$) or Log Dust concentration, smoking.

- FEV1 - Log Endotoxin (EU/m$^3$, pmol/m$^3$) or Log Dust concentration, smoking.

- FEV1/FCV – Log Endotoxin (EU/m$^3$, pmol/m$^3$) or Log Dust concentration, smoking.

- Correlations ($r = 0.15 - 0.24$) and regressions
  - ($R^2 0.02 - 0.12$) weak in all cases

- C12, C14, C18 3OHFAs best models for FVC, FEV1, FEV1/FCV.
Conclusions

- Personal exposures to dust and endotoxin quite variable. Some very high.

- Means exceed current recommended OELs

- Geometric mean dust levels highest among grain elevator operators (4.50 mg/m³) and lowest among farm workers (2.49 mg/m³)

- Geometric mean endotoxin exposure level was highest among feedlot workers (1,093 EU/m³ by rFC).

- Even chain 3OHFA (12, 14, 18) highest in dairy and cattle feedlots.
Conclusions

- 26% of participants had a cross shift drop in FEV1 > 5%. 10% had a drop in FEV1 > 10%.

- 19% had a drop in cross shift drop in FVC exceeding 5%. 8% had FVC decrease > 10%.

- The proportions were largest in farmers, followed by dairy workers, grain handlers, and cattle feedlot workers.
Conclusions

- Smoking, endotoxin/dust 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).

- 3OHFAs, especially C12, C14, C18 were associated with cross shift changes in FVC and FEV1.

- Even chain 3OHFAs (pg/mg) associated with increased PMN, MPO, Albumin, ECP in nasal lavage (not odd chains).
Conclusions

- Respiratory symptoms were similar among those with and without elevated dust/endotoxin exposures, suggesting development of tolerance from subchronic organic dust exposure.

- Acute endotoxin exposure was an important predictor of change in FEV1, and markers of inflammation.

- These results suggest that workers’ exposed to elevated concentrations of endotoxin-containing dusts are more susceptible to acute inflammatory effects of endotoxin containing dusts, and that extended workplace exposures confer a degree of tolerance, or that sensitive workers who do not adapt leave the industry over time.
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