Molds, Mycotoxins and Public Health

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PO Box 2050 Benson, Arizona 85602
Case Study: Tucson Bank, 1994

- **Bank Manager**
  - 49 y.o. Married Female
  - Symptoms
    - 18 month hx arthritis (wheelchair bound)
    - General malaise
    - Excessive fatigue
    - Cognitive dysfunction w/ memory loss
    - Difficulty concentrating
    - Multiple other symptoms

- **9–10 Other Employees**
  - Similar symptoms
    - Shortness of breath
    - Cognitive problems
    - Periorbital edema
    - Skin rashes limited to exposed skin.
    - Workplace related
    - Worsened on Mondays
    - Improvement on weekends & vacations
Case Study: Tucson Bank, 1994

- **Clinical Findings**
  - Urticarial & granulomatous skin changes
  - Small airway obstruction & reactivity
  - Immunologic hyper activation & simultaneous suppression
  - Multiple inflammatory symptoms
  - Elevated EBV IgG antibodies
  - Cognitive deficits

- **Environmental findings**
  - Presence of multiple filamentous molds
  - Pipes leaking in common wall to air handler & bathroom
  - Elevated *Stachybotrys* spore counts detected in:
    - Air and surface
    - Highest on the manager’s desktop
    - All employees passed this desk to go to break room
Hidden Fungi
Historical Perspective

• Biblical Warning
  – *If your house be contaminated with plagues, molds, and Leprosy, put the contents in the middle and set it aflame*  
    --Leviticus

• *Robigalia- April 25th*
  – 7th to 8th Century BC
  – *To protect trees & grains from rusts or mildew*

• *Occupational Disease*
  – *Brown Lung Disease*
  – *Farmer’s Lung*
  – *Pigeon Breeder’s Disease*
  – *Yellow Rice Disease*
Toxic Effects of Mycotoxins in Humans

- **Trichothecenes**
  
  “This family of mycotoxins causes multiorgan effects including emesis and diarrhea, weight loss, nervous disorders, cardiovascular alterations, immunodepression, hemostatic derangements, skin toxicity, decreased reproductive capacity, and bone marrow damage”

- *Medical Aspects of Chemical and Biological Warfare, Chapter 34: Trichothecene Mycotoxins, 1997*
Fungi Producing Trichotheceene Toxins

- Fusarium
- Trichotheceum
- Myrothecium
- Cephalosporium
- Stachybotrys
- Verticimonosporium
- Cylindrocarpon
Trichothecenes

• Major category of mycotoxins
• 1st case of mycotoxicosis
  – 1932
  – USSR
  – Alimentary toxic aleukia
    • 60% mortality
• Major chemical warfare agent
  – Yellow Rain
Fungi Common in Sick Building Syndrome

- Alternaria
- Acremonium
- Epicoccum
- Trichoderma
- Stachybotrys
- Penicillium
Top Twenty Symptoms (N=195)

- Headache
- Intense Fatigue
- Spaciness
- Memory Problems
- Nasal Symptoms
- Throat Discomfort
- Coughing
- Lightheaded
- Watery eyes
- Sinus Discomfort
- Bloating
- Dizziness
- Insomnia
- Joint Discomfort
- Weak Voice
- Rash
- Coordination Probs
- Muscle Spasm
- Slurred/Word Find
- Cold Intolerance

Legend:
- One a Month
- Several Times a Month
- Once a Week
- Several Times a Week
- Daily to Almost Daily
Compared Symptom Profiles

156 Exposed Patient’s Compared to 28 Referent Patient’s Means

- Cold Intolerance
- Muscle Spasm
- Rash
- Coordination problems
- Weak Voice
- Dizziness
- Bloating
- Insomnia
- Joint Discomfort
- Lightheaded
- Throat discomfort
- Watery Eyes
- Sinus Discomfort
- Coughing
- Spaciness
- Memory Problems
- Nasal Symptoms
- Headache
- Fatigue

Non Exposed
Exposed

11/11/02 APHA Philadelphia
Property of ImmunTox, LLC
Historical Perspective

• Spring 1998
  – CDC MMWR 9 cases hemorrhagic pneumonitis in Infants
    • *Stachybotrys chartarum* found in lungs
  – *Stachybotrys chartarum* (atraz) present in homes
  – *In National Survey* Ruth Etzel, MD, Ph.D. finds 115 cases of hemosiderosis associated with mold exposure (mortality approx. 15%)
**Stachybotrys chartarum**

- Isolated from lung of a child with pulmonary hemosiderosis
  - Strain JS5106
  - Stachyrase A
    - New Chymotrypsin-like Serine proteinase
    - Cleaves major protease inhibitors,
    - Several active peptides, and
    - Collagen
    - All above found in the lung
      - *Infection and Immunity, Jan 2002, p 419-423*
Immune Effects

• Increased B-cells
• Excessive T-cell activation
  – CD3+CD26+(TA1) excess
  – CD3+HLA-DR+ excess
• Suppressor cell activation
  – CD8+CD38+ excess
  – CD8+HLA-DR+ excess
  – Increased Interleuken 2 Receptors on T-cells
Exposed Patients to Normal Ranges for Activation Markers

- Exposed Mean
- Min Normal
- Mean Normal
- Max Normal

% CD20+ (B Cells)
% CD5+ CD25+ (IL-2)
% CD3+ CD26+
% CD3+ HLA-DR+
% CD8+ CD38+
% CD8+ HLA-DR+
Immune Depression Effects

%CD3-CD16+CD56+ Natural Killer Cells as % Tot Lymph

%CD8+CD11b+ Human Leuko Compliment Receptors
Immune Suppression

Lymph Stimulation by Phytohemagglutinin (PHA)

Concanavillin Stimulation
Immune Effects

• Increased markers of autoimmunity
  – Anti CNS myelin antibodies
  – Anti PNS myelin antibodies
  – Anti smooth muscle antibodies
  – Anti nuclear antibodies
Immune Effects (n=195)

Autoimmune Antibodies

Patients w/ Pos Autoantibodies of 195 Total Patients
Factors affecting respirability

• Particulate size
  – Greater than 5 microns
    • Non-respirable
    • Except if aspect ratio > 1:3
  – 5 microns to 0.005 micron
    • Considered respirable
    • Reaches alveoli
    • Passive filtration is useless
Pulmonary Effects of Spores
Smokers and Nonsmokers

Degree of Small Airway Obstruction Percentage of Predicted FEF 75%

Critical <30
V Severe 30 - 45
Severe 46 - 55
Moderate 56 - 65
Mild 66 - 80
Normal >80

14
41
35
31
29
29
Pre and Post Bronchodilator FEF 75% Combined Smokers and Non-Smokers
Pre Dilator Degree of Severity
Smokers vs. Nonsmokers

<table>
<thead>
<tr>
<th>Severity</th>
<th>Nonsmokers</th>
<th>Smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Critical &lt;30</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Very Severe 30-45</td>
<td>24</td>
<td>17</td>
</tr>
<tr>
<td>Severe 46-55</td>
<td>14</td>
<td>21</td>
</tr>
<tr>
<td>Moderate 56-65</td>
<td>8</td>
<td>24</td>
</tr>
<tr>
<td>Mild 66-80</td>
<td>4</td>
<td>23</td>
</tr>
<tr>
<td>Normal &gt;80</td>
<td>2</td>
<td>25</td>
</tr>
</tbody>
</table>

Legend:
- Blue: Nonsmokers
- Red: Smokers
Mean Pre and Post FEF 75%  
Nonsmokers vs. Smokers

Pre FEF75%  Post FEF75%

Nonsmokers
Smokers

- Nonsmokers
- Smokers
Pre and Post FEF 75% by Smoke Pack Years

![Bar chart showing Pre and Post FEF 75% by Smoke Pack Years]

- > 20 Years
- 11 to 20
- 6 to 10
- 1 to 5

Legend:
- Pred Pre FEF75%
- Pred post FEF75%
Pulmonary Mycotoxicosis

- **Pulmonary Nodules**
  - Associated with intense chest pain
  - Aspergillus common, but not only cause
  - Presence across fissure line implies “kissing” lesions reflecting exodigestion—secretion of digestive enzymes
  - Antifungals effective, but often induce toxicity (liver, bone marrow, kidneys vulnerable)
Symptom Profiles – Dr. Crago

- Low in energy
- Headaches
- Remembering things
- Trouble conc
- Worrying
- Blank Mind
- Double-check
- Feeling weak
- Feeling tense
- Feeling blocked
- Easily annoyed
- Dif making decisions
- Trouble falling asleep
- Feeling blue
- Slowly for correctness
- Nervousness
- Muscle soreness
- Hot/cold spells
- Tingling
- Loss sexual interest

0 3 6 9 12 15 18 21 24
Kilburn Neurotox Data

• The following data is Dr. K. Kilburn’s analysis of 43 mycotoxin exposed patients and 202 non-exposed referent subjects as percent of predicted (PFTs), means, Standard Deviation (SD), and P values by analysis of variance.
## Demographic Data

<table>
<thead>
<tr>
<th></th>
<th>43 exposed Mean +/- SD</th>
<th>202 referent Mean +/-SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>48.2 +/- 13.9</td>
<td>46.6 +/- 20.6</td>
<td>0.624</td>
</tr>
<tr>
<td>Education (years)</td>
<td>15 +/- 2.6</td>
<td>12.9 +/- 2.3</td>
<td>0.0001</td>
</tr>
</tbody>
</table>
Reaction Times (ms)

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<th>43 exposed Mean +/- SD</th>
<th>202 referent Mean +/-SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple</td>
<td>104.0 +/- 6.6</td>
<td>99.9 +/- 3.7</td>
<td>0.0001</td>
</tr>
<tr>
<td>Choice</td>
<td>102.6 +/- 6.6</td>
<td>100.0 +/- 3.7</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

**Reaction Time**

- **Simple Reaction**
  - Time (ms): 104.0 ± 6.6
  - P = 0.0001

- **Choice Reaction**
  - Time (ms): 100.0 ± 3.7
  - P = 0.0001
### Balance Sway Speed (cm/sec)

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<th>202 referent Mean +/-SD</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>Eyes Opened</td>
<td>140.5 +/- 49.9</td>
<td>100.2 +/- 2.5</td>
<td>0.0001</td>
</tr>
<tr>
<td>Eyes Closed</td>
<td>168.7 +/- 102.7</td>
<td>103.1 +/- 26.7</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

**Graph:**
- **Eyes Opened:** P=0.0001
- **Eyes Closed:** P=0.0001
Blinking Reflex Latency R-1 (ms)

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</thead>
<tbody>
<tr>
<td>Right</td>
<td>113.9 +/- 10.8</td>
<td>99.4 +/- 14.6</td>
<td>0.0001</td>
</tr>
<tr>
<td>Left</td>
<td>115.1 +/- 11.4</td>
<td>96.4 +/- 13.2</td>
<td>0.0001</td>
</tr>
</tbody>
</table>
## Color Perception Score

<table>
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<th>202 referent Mean +/- SD</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>Right</td>
<td>69.6 +/- 40.8</td>
<td>102.6 +/- 51.1</td>
<td>0.0001</td>
</tr>
<tr>
<td>Left</td>
<td>75.8 +/- 48.8</td>
<td>102.6 +/- 51.1</td>
<td>0.002</td>
</tr>
</tbody>
</table>

![Bar chart showing color perception scores for Right and Left eyes, with P values of 0.0001 and 0.002 respectively.](chart.png)

**43 EXPOSED MEAN**

**202 REFERENT MEAN**

*Right P=0.0001, Left P=0.002*
Visual Performance

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<th>P value</th>
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</thead>
<tbody>
<tr>
<td>Right</td>
<td>87.5 +/- 23.9</td>
<td>100.0 +/- 22.8</td>
<td>0.006</td>
</tr>
<tr>
<td>Left</td>
<td>90.0 +/- 20.9</td>
<td>101.1 +/- 21.7</td>
<td>0.008</td>
</tr>
</tbody>
</table>
Grip Strength (lbs)

<table>
<thead>
<tr>
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<th>202 referent Mean +/-SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right</td>
<td>91.6 +/- 20.0</td>
<td>99.3 +/- 17.5</td>
<td>0.012</td>
</tr>
<tr>
<td>Left</td>
<td>86.8 +/- 22.5</td>
<td>99.1 +/- 17.5</td>
<td>0.0001</td>
</tr>
</tbody>
</table>
Verbal Recall

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<thead>
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<th>43 exposed Mean +/- SD</th>
<th>202 referent Mean +/-SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediate</td>
<td>76.7 +/- 23.4</td>
<td>99.8 +/- 31.1</td>
<td>0.0001</td>
</tr>
<tr>
<td>Delayed</td>
<td>68.7 +/- 38.2</td>
<td>99.9 +/- 41.3</td>
<td>0.0001</td>
</tr>
</tbody>
</table>
### Information, Pictures, Similarities

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<th>202 referent Mean +/-SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Information</td>
<td>88.9 +/- 34.7</td>
<td>101.5 +/- 39.4</td>
<td>0.052</td>
</tr>
<tr>
<td>Picture Completion</td>
<td>78.8 +/- 30.7</td>
<td>99.3 +/- 32.2</td>
<td>0.0002</td>
</tr>
<tr>
<td>Similarities</td>
<td>95.5 +/- 25.8</td>
<td>98.1 +/- 41.2</td>
<td>0.691</td>
</tr>
</tbody>
</table>

**Bar Chart:**
- **Information:** P=0.052
- **Picture Completion:** P=0.0002
- **Similarities:** P=0.691
Profile of Mood States

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<th>202 referent Mean +/-SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score</td>
<td>59.7 +/- 41.8</td>
<td>21.0 +/- 31.6</td>
<td>0.0001</td>
</tr>
<tr>
<td>Tension</td>
<td>16.1 +/- 8.5</td>
<td>9.1 +/- 5.8</td>
<td>0.0001</td>
</tr>
<tr>
<td>Depression</td>
<td>14.6 +/- 12.2</td>
<td>8.1 +/- 9.3</td>
<td>0.0001</td>
</tr>
<tr>
<td>Anger</td>
<td>13.4 +/- 10.1</td>
<td>8.3 +/- 7.9</td>
<td>0.0003</td>
</tr>
<tr>
<td>Fatigue</td>
<td>15.2 +/- 7.3</td>
<td>7.6 +/- 6.1</td>
<td>0.0001</td>
</tr>
<tr>
<td>Vigor</td>
<td>10.3 +/- 6.7</td>
<td>18.3 +/- 6.3</td>
<td>0.0001</td>
</tr>
<tr>
<td>Confusion</td>
<td>12.9 +/- 6.9</td>
<td>6.1 +/- 4.5</td>
<td>0.0001</td>
</tr>
</tbody>
</table>
Mechanism for Neurotoxic Effects of Micotoxins

- Fumonisin B1 (FB1) enzyme inhibitor blocking biosynthesis of sphingolipids and accumulations of sphiganine
- Resultant brain hemorrhage (leukoencephalomalacia) \((\text{Wilson, 1992})\)
- Sterigmatocystin interferes with sphingosine metabolism: an essential component of neuronal membranes
- Tremulo- and Vomitoxins are mycotoxins
Fungal Effects

• Neurological Component
  – Cognitive dysfunction
    • Confusion and spaciness
    • Memory loss
    • Spatial disorientation
    • Dyslexia
    • Seizures

• Attention Deficit Disorders
  – Suspect intrusion of paroxysmal increased brain wave frequency and activity
Mycotoxin Effects

- Neurological
  - Behavioral changes
  - Cognitive changes
  - Ataxia
  - Convulsions
  - Multiple sclerosis
  - Optic neuritis
- Immune suppression
- Interferes with protein synthesis

- Pulmonary
  - Small airway obstruction
  - Hypersensitivity pneumonitis
  - Hemorrhagic pneumonitis

- Cardiovascular
  - Vascular permeability
Mycotoxin Intoxication

- Acute and Chronic Mycotoxicosis can cause serious and sometimes multisystem diseases with severe and sometime fatal outcomes.
- The possibility of Mycotoxin intoxication should be considered when an acute disease occurs in several persons when there is no evidence of infection with a known etiologic agent, and no improvement in the clinical picture follows treatment.
Assays for Trichothecenes

• Protein Translation assay in Airborne Particulates
  – Rapid, inexpensive assay using firefly luciferase has been developed to detect and quantify the fungus-derived toxicity of airborne particulates
  – Entire testing procedure can be accomplished in 2 hours
  – Authors demonstrated T-2 toxin, Satratoxin, and DON inhibit luciferase mRNA in a cell free rabbit reticulocyte system
  – Results are highly reproducible
    – Applied and Environmental Microbiology, Jan, 1999, p. 88-94
Symptoms of Sick Building Syndrome

• Head, Eyes, Nose, Throat
  – Headache
  – Dizziness
  – Dry eyes
  – Watery eyes
  – Itchy eyes
  – Epistaxis
  – Stuffy nose
  – Rhinorrhea
  – Alopecia

• Gastrointestinal
  – Nausea & Vomiting
  – Diarrhea
  – Constipation

• Respiratory
  – Cold & flu symptoms
  – Cough
Fungal Exposure & Health Effects

- Pathogenic infections
- Allergic illnesses from fungi
- Hypersensitivity pneumonitis
Conclusion

• Acute and Chronic Mycotoxicosis can cause serious and sometimes multisystem diseases with severe and sometime fatal outcomes.

• The possibility of Mycotoxin intoxication should be considered when an acute disease occurs in several persons when there is no evidence of infection with a known etiologic agent, and no improvement in the clinical picture follows treatment.
Afterword:

Be Not Afraid
To Look For
Zebras

--Anon
Molds, Mycotoxins and Human Health

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