Indoor Toxicity and Fungal Inhalation Risk of Patients and Clinical Findings

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Health and immunology study following exposure to toxigenic fungi (*Stachybotrys chartarum*) in a water-damaged office environment

Received: 3 May 1995/Accepted: 17 October 1995
Fungal Exposure:
Various agents and disease outcomes

**Agents:**
- Allergens
- Ergosterol
- (1-3)-β-D-glucan
- Mycotoxins
- Microbial volatile organic compounds (MVOCs)
- ???

**Allergy + Non-allergic:**
- Dermatitis,
- Urticaria
- Rhinitis, Sinusitis
- Asthma
- Extrinsic allergic alveolitis “humidifier fever”
- Organic dust toxic syndrome
- Toxic – irritant effects

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Diagnostic problems

Exposure
Multiple Mixture
- dose

Non-specific symptoms
Multiple disease endpoints

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Health effects of fungi

- Mycotoxicosis
- Hypersensitivity pneumonitis
- Bronchitis
- Allergic diseases
- Dermatitis; air way infections
- Irritative and non-specific symptoms

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Database

Patients evaluated in occupational and environmental health clinic from December 1999 to February, 2005

* Adult patients (Patients ≥ 18 years at time of visit, exposure duration ~ 2y)
* Advanced environmental testing (including airborne cytotoxicity study MTT)
* Completed self-administered health questionnaire.

Compared to adult clinic patients (controls) without self-reported exposure to dampness/mold at home or at work.
Demographics

Age

Gender - Smoker

Patient: n = 79-94; Control: n = 25-26

Gender (% Female) [***]

Smoking status

*** = p < 0.001
Symptom complex

Upper respiratory [***]
Lower respiratory [***]
CNS [***]
Allergy, prev.

Patient: n = 95; Control: n = 26

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Pulmonary function test abnormalities

Patient: n = 20

- Combined (O) + (S-A)
- Combined (R) + (S-A)
- Small airway (S-A)
- Restrictive (R)
- Obstructive (O)

% Yes

0 10 20 30 40 50 60 70
Patient’s IgG Antibody response and comparison with environmental sampling identification

Patient: n = 66 - 69

- **Alternaria * IgG**
  - Alternaria

- **Aspergillus * IgG**
  - A. fumigatus

- **Eurotium * IgG**
  - A. fumigatus

% IgG MSAB Elevated

<table>
<thead>
<tr>
<th>Fungi reported: YES</th>
<th>Fungi reported: NO</th>
</tr>
</thead>
</table>

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Patient’s IgG Antibody response and comparison with environmental sampling identification

Patient: n = 66 - 69

- Penicillium * IgG
- Penicillium
- Phoma * IgG
- Phoma
- Stachybotrys * IgG
- Stachybotrys
- Trichoderma * IgG
- Trichoderma

% IgG MSAB Elevated

Fungi reported: YES
Fungi reported: NO
Patient’s IgE Antibody response and comparison with environmental sampling identification

Fungi reported: YES
Fungi reported: NO

% IgE MSAB Elevated

Patient: n = 63 - 67
IgE Mold Specific Antibodies & Environmental Exposure

Patient: n = 63 - 67

Fungi reported: YES
Fungi reported: NO

% IgE MSAB Elevated

- Eurotium * IgE
- Aspergillus
- Stachybotrys * IgE
- Stachybotrys

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IgA Mold Specific Antibodies & *Stachybotrys chartarum* exposure

![Graph showing IgA MSAB Elevated with Fungi reported: YES and NO categories.]

Patient: n = 68
<table>
<thead>
<tr>
<th>Agent – Serology</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>+ predictive value</th>
<th>- predictive value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. fumigatus IgE</td>
<td>0.04</td>
<td>0.91</td>
<td>0.93</td>
<td>0.85</td>
</tr>
<tr>
<td>S. chartarum IgE</td>
<td>0.05</td>
<td>0.94</td>
<td>0.6</td>
<td>0.33</td>
</tr>
<tr>
<td>Alternaria a. IgG</td>
<td>0.22</td>
<td>0.74</td>
<td>0.53</td>
<td>0.42</td>
</tr>
<tr>
<td>Penicillium n. IgG</td>
<td>0.25</td>
<td>0.77</td>
<td>0.95</td>
<td>0.08</td>
</tr>
<tr>
<td>A. fumigatus IgG</td>
<td>0.24</td>
<td>0.83</td>
<td>0.17</td>
<td>0.88</td>
</tr>
<tr>
<td>S. chartarum IgG (adults only)</td>
<td>0.09</td>
<td>0.85</td>
<td>0.48</td>
<td>0.37</td>
</tr>
<tr>
<td>S. chartarum IgG (children only)</td>
<td>0.07</td>
<td>1</td>
<td>1</td>
<td>0.26</td>
</tr>
<tr>
<td>Trichoderma v. IgG</td>
<td>0.28</td>
<td>0.65</td>
<td>0.22</td>
<td>0.71</td>
</tr>
</tbody>
</table>
Indoor environments of 55 patients (1999 and 2005) with verified moisture related building damage and indoor fungal growth were studied. In total, 161 high-volume air samples were analyzed for trichothecene (Roridin A) content by the ELISA method and fungi in this comparison.
Air sampling (24 h)

Mycology

with special attention to
Stachybotrys ch.

Inhalation Exposure - Logistics and Methodology

Clinical data

Case

Bulk samples

Mycology

Toxicity

Mycotoxins

Mycotoxin analysis

HPLC-DAD

GC-MS

EIA

Cytotoxicity screening of crude extracts (MTT-test)

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paper in preparation
Airborne Cyto-Toxicity Results:

Samples

High (IC50 =< 31.25)

Moderate (31.25 < IC50 =< 125)

Low toxicity (IC50 > 125)

% (n = 203)

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## Airborne Cytotoxicity & Viable Fungi

<table>
<thead>
<tr>
<th>Viable fungi (% yes, (n))</th>
<th>RoA Elisa (ng/g)</th>
<th>Spearman's</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>level</td>
<td>&lt; 2</td>
</tr>
<tr>
<td><strong>Acremonium sp.</strong> (+)</td>
<td>4.0 (1)</td>
<td>4.0 (1)</td>
</tr>
<tr>
<td>+</td>
<td>8.0 (2)</td>
<td>8.0 (2)</td>
</tr>
<tr>
<td>++</td>
<td>8.0 (2)</td>
<td>4.0 (1)</td>
</tr>
<tr>
<td>+++</td>
<td>0.0 (0)</td>
<td>4.0 (1)</td>
</tr>
<tr>
<td><strong>Alternaria sp.</strong> (+)</td>
<td>7.7 (1)</td>
<td>no data</td>
</tr>
<tr>
<td>+</td>
<td>15.4 (2)</td>
<td>no data</td>
</tr>
<tr>
<td>++</td>
<td>15.4 (2)</td>
<td>no data</td>
</tr>
<tr>
<td><strong>Aspergillus sp.</strong> (+)</td>
<td>2.0 (2)</td>
<td>2.0 (2)</td>
</tr>
<tr>
<td>+</td>
<td>12.9 (13)</td>
<td>15.8 (16)</td>
</tr>
<tr>
<td>++</td>
<td>8.9 (9)</td>
<td>5.9 (6)</td>
</tr>
<tr>
<td>+++</td>
<td>5.9 (6)</td>
<td>1.0 (1)</td>
</tr>
<tr>
<td><strong>Chaetomium sp.</strong> (+)</td>
<td>5.9 (1)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>+</td>
<td>0.0 (0)</td>
<td>5.9 (1)</td>
</tr>
<tr>
<td>++</td>
<td>11.8 (2)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>+++</td>
<td>11.8 (2)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td><strong>Cladosporium sp.</strong> (+)</td>
<td>1.4 (1)</td>
<td>1.4 (1)</td>
</tr>
<tr>
<td>+</td>
<td>23.0 (17)</td>
<td>18.9 (14)</td>
</tr>
<tr>
<td>++</td>
<td>6.8 (5)</td>
<td>2.7 (2)</td>
</tr>
<tr>
<td>+++</td>
<td>0.0 (0)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td><strong>Paecilomyces sp.</strong> (+)</td>
<td>0.0 (0)</td>
<td>3.6 (1)</td>
</tr>
<tr>
<td>+</td>
<td>32.1 (0)</td>
<td>10.7 (3)</td>
</tr>
<tr>
<td>++</td>
<td>3.6 (1)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td><strong>Penicillium sp.</strong> (+)</td>
<td>10.8 (12)</td>
<td>14.4 (16)</td>
</tr>
<tr>
<td>+</td>
<td>14.4 (16)</td>
<td>4.5 (5)</td>
</tr>
<tr>
<td>++</td>
<td>9.0 (10)</td>
<td>2.7 (3)</td>
</tr>
</tbody>
</table>
Cytotoxicity Testing of Filter Papers from 24 h Air Sampling

Inhalation Exposition - Results

Stachybotrys chartarum

<table>
<thead>
<tr>
<th>n cases</th>
<th>Stachybotrys chartarum</th>
<th>Macrocyclic Trichothecenes</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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Fungal toxicity and neurocognitive dysfunction
(W. Gordon, PhD et al)

• 22 neurocognitive cases selected that included indoor air toxicity assessments
Brain Injury Screening Questionnaire (BISQ) Results:

Table 2. BISQ Symptom Report: Means and Standard Deviations and Results of ANOVAs Comparing Mean Numbers of BISQ Symptoms Between Groups

<table>
<thead>
<tr>
<th>Type of Symptoms</th>
<th>Mold(^a)</th>
<th>Mild TBI(^b)</th>
<th>Moderate TBI(^c)</th>
<th>No Disability(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
<td>M</td>
<td>SD</td>
</tr>
<tr>
<td>Physical</td>
<td>6.07</td>
<td>3.73</td>
<td>7.11</td>
<td>4.88</td>
</tr>
<tr>
<td>Cognitive</td>
<td>18.67</td>
<td>11.06</td>
<td>21.25</td>
<td>14.59</td>
</tr>
<tr>
<td>Behavioral</td>
<td>8.13</td>
<td>6.82</td>
<td>11.89</td>
<td>8.20</td>
</tr>
<tr>
<td>All</td>
<td>32.87</td>
<td>19.37</td>
<td>40.25</td>
<td>26.17</td>
</tr>
<tr>
<td>25 S&amp;S(^b)</td>
<td>10.10</td>
<td>6.23</td>
<td>8.72</td>
<td>6.38</td>
</tr>
</tbody>
</table>

Note. BISQ = Brain Injury Screening Questionnaire; ANOVA = Analyses of Variance; TBI = traumatic brain injury.
\(^{a}n = 30. ^{b}n = 65. ^{c}n = 26. ^{d}n = 47. ^{S&S}S = symptoms sensitive and specific to TBI (Gordon et al., 2000).

Patients with (toxigenic) indoor mold exposure history and traumatic brain injury report similar symptoms and problems.
Neurocognitive Testing Results, WMS III, Airborne Toxicity Findings

<table>
<thead>
<tr>
<th>Test</th>
<th>Moderate-high, n = 11</th>
<th>No, n = 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>WMS-III, Visual Immediate Memory Index</td>
<td>Moderate</td>
<td>No</td>
</tr>
<tr>
<td>WMS-III, Auditory Immediate Memory Index</td>
<td>Moderate</td>
<td>No</td>
</tr>
<tr>
<td>WMS-III, Working Memory Index</td>
<td>Moderate</td>
<td>No</td>
</tr>
<tr>
<td>WMS-III, General Memory Index</td>
<td>Moderate</td>
<td>No</td>
</tr>
</tbody>
</table>

Of 22 neurocognitive cases selected that included indoor air toxicity assessments
Of 22 neurocognitive cases selected that included indoor air toxicity assessments.
Of 22 neurocognitive cases selected that included indoor air toxicity assessments

WMS-III, General Memory Index
WMS-III, Working Memory Index
WMS-III, Auditory Immediate Memory Index (*)

* = p < 0.05

Moderate to high toxicity (n = 11)
No toxicity (n = 9)

% Equal or below 16th percentile

Neurocognitive Testing Results, WMS III, Toxicity, % Reduced functioning (≤ 16th percentile)
Neurocognitive Testing Results, WMS III, Toxicity, % Reduced functioning (≤ 16th percentile)

- WMS-III, Visual Immediate Memory Index
- WMS-III, Immediate Memory Index
- WMS-III, Auditory Delayed Memory Index
- WMS-III, Visual Delayed Memory Index
- WMS-III, Auditory Recognition Index

% Equal or below 16th percentile

• 22 neurocognitive cases selected that included indoor air toxicity assessments
Reviews and committee papers – who are the reviewer and what are their motives?

• ACOEM – “Mold - Evidence Paper”
  – American College of Occupational and Environmental Medicine (10/2002)
    – Authors: Hardin, Kelman, Saxon
    – See also: Manhattan Institute (same content) (7/03)

• IOM - Damp Indoor Spaces and Health
  – Institute of Medicine (2004)

• Kuopio Finland Toxic Mold Meeting (7/2004)
  – ISIAQ

• Norddamp (Bornehag et al, 2004)
Critique of “evidence Papers”

- The motives and intentions of the authors have been examined and questioned:
  - “A Critique of the ACOEM Statement on Mold: Undisclosed Conflicts of Interest in the Creation of an “Evidence-based” Statement”
    - By JAMES CRANER in INT J OCCUP ENVIRON HEALTH 2008;14:283–298
  - “Court of Opinion: Amid Suits Over Mold. Experts Wear Two Hats; Authors of Science Paper Often Cited by Defense Also Help in Litigation”
  - “Position paper on molds by AAAAI is seriously flawed”.
    - Letters to editor of journal by different authors see: J ALLERGY CLIN IMMUNOL VOLUME 118, NUMBER 3
Detection of Airborne *Stachybotrys chartarum* Macrocyclic Trichothecene Mycotoxins in the Indoor Environment

T. L. Brasel, J. M. Martin, C. G. Carriker, S. C. Wilson, and D. C. Straus*

Department of Microbiology and Immunology, Texas Tech University Health Sciences Center, Lubbock, Texas 79430

Received 9 March 2005/Accepted 12 July 2005

allergens Can f 1, Der p 1, and Fel d 1. For test buildings, the results showed that detectable toxin concentrations increased with the sampling time and short periods of air disturbance. Trichothecene values ranged from \(<10\) to \(>1,300\) pg/m\(^3\) of sampled air. The control environments demonstrated statistically significantly \((P < 0.001)\) lower levels of airborne trichothecenes. ELISA specificity experiments demonstrated a high specificity for the trichothecene-producing strain of *S. chartarum*. Our data indicate that airborne macrocyclic trichothecenes can exist in *Stachybotrys*-contaminated buildings, and this should be taken into consideration in future indoor air quality investigations.
Conclusions

• Patient IgE and IgG show limited correlation with specific environmental findings (low sensitivity, good specificity)
• Patient show (also) non IgE- or IGG-mediated or associated exposure effects
• New onset of symptoms and abnormalities in non-sensitized patients (new onset Dx)
Conclusions

• Cognitive impairment symptoms similar to patients with traumatic brain injury (TBI)
• Airborne (fungal) toxicity appear to be correlated with some neurocognitive dysfunction
• Improved, specific exposure data necessary to improve environmental/occupational diagnosis
• Mycotoxin body burden indicator needed to validate study findings
Conclusions

• Airborne Satratoxins (macrocyclic trichotheccenes)
• Detection of mycotoxins in air samples not or weakly correlated with fungal spores
• S. chartarum not necessarily correlated with the presence of satratoxins
• Other cytotoxic compounds could be detected by use of the bioassay
• Methods appears to be reliable to differentiate between cytotoxic and non-cytotoxic filter papers, i.e. toxic and non-toxic environments
“Wissen ist nicht genug – wir müssen handeln”

“Knowing is not enough; we must apply.”
- J. W. Goethe

Child with asthma in Spanish Harlem, N.Y. ...
Johanning et al; EHP 1999;107 (3)
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