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Long-term Sampling Reveals the Beneficial Role of Fungi in Allergic Sensitization of Children

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by

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Abstract

Indoor air quality has become increasingly important as we live in a society where the majority of our time is spent indoors. Specific attention has been drawn to airborne fungal spores as a factor affecting indoor air quality. This study targeted shortcomings of other studies by utilizing long-term sampling and total fungal spore enumeration to determine associations between health outcomes and fungal spore concentrations, and between visible mold and air concentrations. In this study, fungal spore samples were collected using a Button Personal Inhalable Sampler (SKC, Inc.) for 48 hours at a flow rate of 4 L min⁻¹. Sampling was conducted in 145 homes in the spring (March-May) or the fall (August-October). Fungal spores were analyzed using microscopy-based total counting and identified to the genus or group level. Homes were classified in an onsite home evaluation as moldy or non-moldy based on visible mold and moldy odor. Total spore and individual genus concentrations were analyzed for associations with rhinitis and positive skin prick test results (SPT+). Overall, concentrations varied widely, between < 2 and 2,294 spores m⁻³. No association was observed between visible mold and airborne concentrations. No relationship was observed with SPT(+) and total fungal counts. A non-significant trend was observed between total fungal spore count and the reporting of rhinitis in children (p=0.11). Several significant associations, including inverse associations, were observed, however, when analysis was conducted on the various mold genera and health outcomes. Positive associations were observed between: Basidiospores and rhinitis (p<0.01); *Penicillium/Aspergillus* and SPT(+) to any allergen (p=0.01); *Alternaria* and SPT(+) to any allergen (p<0.01); Inverse associations were observed between: *Cladosporium* and SPT(+) to any allergen (p<0.05) and *Cladosporium* and SPT(+) to aeroallergens (p=0.03). This study indicates that health outcome may vary by mold genera, such that some mold types may have more sensitizing effects, while others may play a beneficial role.
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# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>List of Abbreviations</td>
<td>v</td>
</tr>
<tr>
<td>List of Figures and Tables</td>
<td>vi</td>
</tr>
<tr>
<td>Chapter 1</td>
<td>1</td>
</tr>
<tr>
<td>Background and Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Chapter 2</td>
<td>5</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>5</td>
</tr>
<tr>
<td>Results</td>
<td>11</td>
</tr>
<tr>
<td>Discussion</td>
<td>14</td>
</tr>
<tr>
<td>References</td>
<td>17</td>
</tr>
<tr>
<td>Appendices</td>
<td>23</td>
</tr>
<tr>
<td>Appendix A: Frequency Distributions for Fungal Spore Concentrations by Season, Total Concentration, and Mold Genera</td>
<td>24</td>
</tr>
<tr>
<td>Appendix B: SPSS Data – Descriptive Stats for 3 Season Categories, Total Concentration, and Four Most Frequent Mold Genera</td>
<td>30</td>
</tr>
<tr>
<td>Appendix C: SPSS Data – ANOVA Scheffe Tests for Seasonal Differences</td>
<td>33</td>
</tr>
<tr>
<td>Appendix D: Descriptive Stats for Significant and Borderline Significant Associations</td>
<td>34</td>
</tr>
<tr>
<td>Appendix E: Univariate GLM – Significant and Borderline Significant Associations Health Outcomes Total Concentrations, and Four Most Frequent Mold Genera Controlled for Season</td>
<td>41</td>
</tr>
<tr>
<td>Appendix F: Crosstabular Chi Square – Significant and Borderline Significant Associations Between Health Outcomes and Less Frequent Mold Genera</td>
<td>45</td>
</tr>
</tbody>
</table>
List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>400x</td>
<td>Magnified 400 times the actual size</td>
</tr>
<tr>
<td>600x</td>
<td>Magnified 600 times the actual size</td>
</tr>
<tr>
<td>95% CI</td>
<td>95% confidence intervals</td>
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<tr>
<td>c²</td>
<td>Chi Square test</td>
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<tr>
<td>CCAAPS</td>
<td>Cincinnati Childhood Allergy and Air Pollution Study</td>
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<tr>
<td>GM</td>
<td>Geometric mean</td>
</tr>
<tr>
<td>L/min</td>
<td>Liters per minute</td>
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<tr>
<td>LOD</td>
<td>Limit of detection</td>
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<tr>
<td>m</td>
<td>Meter</td>
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<tr>
<td>MCE</td>
<td>Membranes of cellulose ester</td>
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<tr>
<td>μL</td>
<td>Microliter</td>
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<td>mL</td>
<td>Milliliter</td>
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<tr>
<td>mm</td>
<td>Millimeter</td>
</tr>
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<td>Rhinitis(-)</td>
<td>Negative rhinitis diagnosis</td>
</tr>
<tr>
<td>Rhinitis(+)</td>
<td>Positive rhinitis diagnosis</td>
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<tr>
<td>Spores/m³</td>
<td>Spores per cubic meter of air</td>
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<tr>
<td>SPT</td>
<td>Skin prick testing</td>
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<tr>
<td>SPT(+)</td>
<td>Positive skin prick test to any allergen</td>
</tr>
<tr>
<td>SPT(-)</td>
<td>Negative skin prick test to any allergen</td>
</tr>
<tr>
<td>SPT(+) aero</td>
<td>Positive skin prick test to aeroallergens</td>
</tr>
<tr>
<td>SPT(-) aero</td>
<td>Negative skin prick test to aeroallergens</td>
</tr>
<tr>
<td>SPT(+) mold</td>
<td>Positive skin prick test to mold</td>
</tr>
<tr>
<td>SPT(-) mold</td>
<td>Negative skin prick test to mold</td>
</tr>
</tbody>
</table>
List of Figures and Tables

Figure 1  Association Between Basidiospore Concentrations and Rhinitis (Any Rhinitis) ................................................................. 19

Figure 2  Association Between Penicillium/Aspergillus type spores and SPT(+) to Any Allergen ................................................................. 19

Figure 3  Inverse Association Between Cladosporium sp. and SPT(+) to Any Allergen ................................................................. 20

Figure 4  Inverse Association Between Cladosporium sp. and SPT(+) to Aeroallergens ................................................................. 20

Table 1  Frequency of Mold Genera ................................................................. 21

Table 2  Geometric Mean (GM) of Total Concentration and Four Most Frequent Mold Genera by Season ................................................................. 22

Table 3  Selected Associations Among SPT and Rhinitis and Fungal Spore Concentrations ................................................................. 22
Chapter One

Background and Introduction

In a society where we spend the majority of our time inside, the effect of indoor air contaminants on human health has become increasingly important. Of particular interest is the health outcome that may result from exposure fungi, as well as other microbial contaminants. This interest is largely based on research that has determined that exposures to biological agents in the occupational environment and in the residential indoor environment are associated with adverse health effects that have broad public health implications (Douwes et al., 2002b). There is increasing evidence that mold growth indoors is a risk factor for the development of childhood asthma and allergies (Etzel and Rylander, 1999). Small children have become the focus of many studies on asthma and allergies because they have a higher incidence of allergies than adults, and they spend the majority of their time in the home (Bomchag et al., 2004). Early childhood exposure to fungi may be important for many reasons. A natural stimulation of the immune system of children is required for proper immune system development. However, exposure to mold at a young age may disturb this maturation of the system and increase the risk for reactions to inhaled antigens and irritants in the environment (Rylander and Etzel, 1999).

According to published data, the majority of identified fungal species have been characterized as potential allergens, and exposure to airborne fungi may provoke immune responses. Previous studies have found associations between skin sensitivity and exposure to fungal mycelial fragments and fungal spores using skin prick testing (SPT) (Fadel et al., 1992), and have determined that allergen-specific IgE leads to skin reactivity (Yazdanbakhsh et al., 2002). Bobbitt et al., (2005) reported a strong correlation among atopy, mold sensitization, and sensitization to specific molds that were identified in the patient’s environmental report. Furthermore, an increase in the relative risk of mold sensitization in symptomatic children with
allergic rhinitis has been observed utilizing percutaneous skin testing. It has also been
determined that patients who are sensitized to mold may display a SPT(+) to more than one mold
species. It is thought that this may be caused by independent sensitization to many fungal
species or by allergen cross-reactivity produced by many fungi (Kidon et al., 2004). It has also
been found that children who were sensitized to mold were also more likely to be sensitized to
other aeroallergens (Kidon et al., 2004).

Several studies have found an association between moisture and/or visible mold with the
reporting of rhinitis or rhinitis symptoms (IOM, 2004), and the mechanisms by which mold and
home dampness are associated with rhinitis and respiratory symptoms are apparently numerous
(Kilpeläinen et al., 2002). Additionally, fungi are frequently found in nasal secretions of patients
diagnosed with chronic rhinitis (IOM, 2004). Allergic fungal rhinitis is a disease that results
from a hypersensitivity reaction when fungi attempt to colonize the paranasal sinuses (Palacio et
al, 1997).

Most studies have reported that moisture and/or visible mold increases the risk of respiratory
diseases and symptoms. In addition to the reports of the more commonly associated health
effects (i.e. asthma, allergies, and wheezing), some studies have shown that exposure to fungal
spores may actually decrease the risk of developing atopic asthma in adults (Eduard et al., 2004).
Similar trends have been observed with bacterial endotoxins. This inverse relationship of
microbial exposures and health outcomes has been coined the “hygiene hypothesis” and has
shifted the attention from the adverse health effects to the potential beneficial effects of exposure
to microbial agents (Martinez, 1999). It is believed that microbial exposures (particularly
endotoxin exposure) early in life may actually protect from the development of atopy and
allergic asthma, although the mechanisms for such beneficial effects are not well understood (Douws et al., 2002a).

While most studies have utilized the practice of culture-based sampling and analysis, there are some shortcomings in using this method. Culturable count is only an indicator of the number of viable, or “live” fungal spores that are present within the space. Although this method is useful when identifying spores to the species level, it does not account for the airborne mold spores that are present, but not culturable. Even non-culturable fungal spores contain allergens, toxins, and (1-3)-β-D-glucans, which may cause adverse health effects. In other words, this method has the potential to grossly underestimate actual airborne fungal spore concentrations, and it doesn’t include the quantification of fungal fragments such as hyphal structures, although these may also have toxic or allergenic properties (Douws et al., 2002b; Gómy et al., 2002). This may be a significant oversight when considering the capability of fungal spores to cause allergic sensitization in young populations. Additionally, some practical drawbacks to the method exist. Culture-based samples must be incubated for a period between two and ten days before results can be ascertained (Baxter et al., 2005). Furthermore, certain mold genera such as those that have been labeled as indicator mold types (e.g. *Stachybotrys*) require specific growth conditions, including temperature, humidity, and nutrients. The type of agar media that is used during culture-based enumeration may be more favorable for certain fungal genera, and may, therefore, inhibit the growth of others.

Microscopy-based enumeration is an alternative exposure assessment method. In this method, airborne fungal spores are collected in either impactors, impingers, or filtering devices and all spores are counted under a microscope regardless of their culturability. This method gives the total fungal spore count and is believed to better represent the airborne mold spore
concentrations to which children might be exposed. Additionally, this method allows analysis of
the samples immediately.

Air samples for fungal spores are typically collected during a short sampling interval of five to
ten minutes. Because of this short sampling duration, the peaks and valleys inherent in the
fluctuation of airborne fungal spore concentrations are not captured. Therefore, through chance,
concentrations captured at one point in time may be significantly different from concentrations
measured in the same location at another time. Concentrations may vary because of varied air
currents in the sampling space, fluctuations in humidity, and gravitational settling of the spores.
This problem can be resolved by extending the sampling period. Sampling for a duration of
several days will give an average concentration during that sampling period, regardless of the
peaks and valleys that may occur. This average concentration may be more beneficial in
diagnosing or characterizing the indoor air of the space and could provide further insight into the
concentrations that occupants of that space would typically be exposed to over an extended
period of time.

While several studies have shown a positive association between visible mold and health
outcome (IOM, 2004), previous studies have found little or no association between airborne
fungal spore concentrations and health outcomes. This may be because of the limitations of the
sampling and analytical methods that were used in the previous studies, given that culture-based
analytical techniques were used and that the sampling duration was too short to account for
concentration variability. In this study, the relationship between airborne fungal spore
concentrations, visible mold, rhinitis and SPT(+) were investigated by utilizing a long-term
sampling and total fungal spore enumeration.
Chapter Two

Materials and Methods

Cohort

This study is part of the Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS). All families in the CCAAPS study were enrolled from early 2002 through 2003. The CCAAPS study design includes a subgroup of children in a nested case-control study (atopic children and matched controls), all of whom have biannual air sampling conducted in their homes. For this study, all subjects were between the ages of 1 and 3 at the time of the home visit.

Visible Mold

Associations between visible mold and total fungal spore concentration were examined in this study. Visible mold was assessed during an onsite home visit and identified using surface sampling methods for fungi. The average age of the child at the time of the onsite visit was 8 months. The presence of visible mold was ranked into three separate categories based on the area of visible mold, as well as the home’s history of mold or water damage (Cho et al., 2005).

Air Sample Collection

Bioaerosol samples were collected using a Button Personal Inhalable Aerosol Sampler (SKC, Inc. Eighty-four, PA). The sampler collects inhalable particles (99% collection efficiency for 1μm diameter particles) (Burton, et al., 2005) onto a 25mm polycarbonate membrane filter and 3μm pore size (GE Osmonics, Inc., Minnetonka, MN). This sampler is also used in the outdoor air monitoring station of the CCAAPS study (Adhikari et al., 2003). Samples were collected in 145 homes. Some homes were sampled more than once, and the number of sampling sessions at
each home is dependent on the family’s enrollment time in the case-control study. However, for this study, we only used the sampling session that was closest to the child’s first physician’s office visit when conducting statistical analysis for the health outcomes. A mean of 234 days (median = 190 days) passed between the child’s first clinic visit and the first sampling session in the child’s home. Samples were collected in the spring during the tree pollen season, and in the fall during the ragweed pollen season and the high humidity period. This study includes the first two years of sampling data and thus, the samples were collected in 2003 and 2004.

Indoor stationary samples were collected in rooms that the child’s parent reported as the primary activity room. Areas selected were most typically located in the family room of the home. The parent or caregiver was asked to relocate the sampler to the child’s bedroom when the child was sleeping. The sampler was placed in a small noise-insulated carrying box (approximately 11”x 8”x 10”). A questionnaire was utilized at the conclusion of the sampling session in order to determine the approximate amount of time that the sampler was in the same room as the child. It was determined that the average time the child spent in the home during the 48 hour sampling session was 39 hours (81%), and during those 39 hours, the sampler was in the same room as the child for 35 hours (73%).

Outdoor samples were collected at the CCAAPS study outdoor air sampling station daily during the indoor sampling sessions. The station is centrally located on the rooftop of a two-story office building located approximately three miles north of downtown Cincinnati. The rooftop was approximately 7 m above the ground. Additionally, vegetation in the area of the building was sparse and there were no other large buildings in the area. This allowed for free movement of wind through the area (Adhikari et al., 2003). All outdoor air samples were collected at a flow rate of 4 L/min for a period of 24 hours, while all indoor samples were collected at a flow rate of
4 L/min for a period of 48 hours. All air sampling pumps were calibrated before and after the sampling period using a Bios DryCal® DC-Lite Calibrator (SKC, Inc.).

Sample Preparation

Following the sampling period, filters were removed from the sampler. The filter samples were then extracted into 2 mL of phosphate buffer. An 800 μL aliquot of the sample was made available for the analysis of total fungal spore counts. Samples were prepared for the analysis by filtering 800 μL of the suspension through a 13 mm diameter MCE filter. The filter was placed onto a slide and allowed to completely dry. During the drying process the filter was isolated in a covered petri plate. Once the filter was dry, the filter was made clear by treating the filter with acetone vapor using a modified acetone vaporizing unit. The samples were then mounted with glycerin jelly and covered with a cover slip. The cover slip was sealed using transparent nail enamel.

Sample Analysis

All air samples were analyzed using a bright light microscope (Olympus CX31; Olympus, City, state) at a magnification of 600x or 400x. Analysis was conducted by either examining 40 randomly selected fields of view or by counting 400 particles, whichever was to come first. Fungal spores were identified morphologically as described in Adhikari et al. (2003). All fungal spores were identified to the genus level when possible. If the identification of the fungal spore was unknown, the spore was listed as an unidentified spore. Background debris on the sample was identified and recorded. Undercounting of small and hyaline spores may occur in samples containing high amounts of background debris. Therefore, phase contrast was used to identify hyaline spores. Fungal spore concentration was calculated by:

\[ C_{\text{button}} = \frac{N_{\text{total(button)}}}{(F \times t)} \]  

(1)
where $N_{\text{total}}$ is the total fungal spore count on the filter, $F$ is the average flow rate, and $t$ is the sampling time (Adhikari et al., 2003). The limit of detection (LOD) was 2 spores per cubic meter of air.

**Health Outcomes**

Airborne fungal spore concentrations were examined to determine if there was an association between concentrations and health outcomes of rhinitis and allergen sensitization (SPT+).

Information on rhinitis within the cohort was obtained by administering a symptom questionnaire to the parent of the child. The questionnaire was administered to the parent during the child’s first physician’s office visit. The average age of children at the time of the first clinic visit was 13 months. Rhinitis symptoms were divided into three categories: allergic rhinitis, atopic rhinitis, or any rhinitis. Allergic rhinitis was defined as rhinitis with a positive SPT to any aeroallergen. Atopic rhinitis was defined as rhinitis with a positive SPT to any allergen, including food. The category of any rhinitis included all children who were diagnosed with rhinitis regardless of the SPT results.

SPT were administered during the child’s first doctor’s office visit for food (milk and egg) as well as 15 aeroallergens (Meadow grass, Timothy grass, White Oak, Maple Mix, American Elm, Red Cedar, Short Ragweed, *Alternaria, Aspergillus fumigatus*, *Penicillium* mix, *Cladosporium*, cat, dog, German Cockroach, and house dust mite). With SPT, the allergen was introduced into the superficial epidermis and observed for local reactions. Children who showed a positive reaction (> 3 mm wheel, greater than saline control) to any of the SPT were classified as sensitized. For this study, three outcomes of SPT results were evaluated: SPT(+) = positive SPT to any allergen, SPT(+) aero = positive SPT to aeroallergens only, and SPT(+) mold = positive SPT to mold.
Statistical analysis

Descriptive statistics of each fungal genera and total fungal spore concentrations were obtained after the log transformation was applied to approximate normality as judged by Kolmogorov-Smirnov tests. These included the geometric mean (GM) and 95% confidence intervals (95% CI) for characterizing averages and expected ranges of mean values of fungal spore concentrations. Log-transformed data were tested for seasonal differences using analysis of variance and student’s t-test where seasons were combined if the average concentrations were not significantly different, in order to determine if the statistical analyses of health outcomes would need to be controlled for such differences. Health outcomes were rhinitis symptoms and SPT sensitivity.

All analyses were controlled for season as an association between airborne fungal spore concentration and season was observed. Total concentrations of airborne fungi as well as concentrations of each mold genera were evaluated for associations between concentrations and health outcomes. Fungal concentrations were used as the independent variable in data analyses. All statistical analyses were conducted using SPSS software, version 12.0 for Windows.

Prior to data analysis, it was discovered that many of the values for the individual mold genera were below the LOD and were recorded as zero. Between 10% and 30% of the four most frequent mold types were observed as having values below the LOD, and between 40% and 80% of the samples for the less frequent mold types were observed as having values below the LOD (Table 1). In order to apply the log-transformation a value of 1 was added to all concentration values before analysis (and deducting 1 from the result obtained) (Eudney et al., 1995). The total concentration and four most frequent mold genera (observed in 70% or more of the homes) were
modeled continuously as independent variables in separate logistic regression models and adjusted for season in order to determine if there were any associations to health outcomes. For the mold genera that occurred in 20% or more of the homes, concentrations were ranked categorically as either below the LOD or above the LOD and the analysis was then conducted using a crosstabular Chi Square ($\chi^2$) test to evaluate associations with health outcomes. Unidentified spores and mold types that occurred in less than 20% of the homes were not evaluated. Only the dataset closest to the child’s first physician’s visit was used ($n = 145$).

Differences among mean values of fungal spore concentrations and visible mold categories were examined by analysis of variance. Log-transformed values of the total concentration and the four highest occurring mold types were analyzed. A crosstabular $\chi^2$ test was used to examine associations between visible mold and less frequent mold spores that were ranked categorically. All data available were used to analyze these associations ($n = 288$).
Results

Frequency of Mold Genera

Table 1 shows the frequency of mold genera in the indoor and outdoor environment as well as in the outdoor environment. In addition, the frequencies of mold genera in the sub-dataset for the sampling conducted closest to the child’s first physician’s office visit are included in column 2. The most frequently occurring mold types were Cladosporium sp., Aspergillus/Penicillium type spores, Basidiospores, and Ascospores. These four types of mold were observed in 70% or more of the homes. Six other mold types occurred in 20% or more of the homes, and included Smuts/Myxomycetes, Ganoderma, Alternaria sp., Pitiozymes sp., Epicoccum sp., and those spores that were unidentified. While unidentified spores occurred in 46% of the homes, it should be noted that concentrations of unidentified spores were very low and contributed only between <1% and 13% (mean = 2%) to the total concentration in their respective samples. Outdoor air data showed the same dominant mold genera as the indoor sampling data.

Seasonal Variations

Table 2 shows geometric means and ranges of seasonal concentrations for the fungal spore data in indoor and outdoor air. Total fungal spore concentrations in 288 indoor air samples ranged between < 2 and 2,295 spores/m³ (GM = 159). Each sampling session (spring 2003, fall 2003, spring 2004, fall 2004) was also evaluated to determine if it was different from the other three sampling sessions. It was discovered that only the spring 2004 sampling period was different from the other sampling sessions (p < 0.001). This difference was most likely caused by the variation in the outdoor air concentration as the outdoor sampling data displayed similar seasonal trends as the indoor sampling data. Therefore, fall 2003 and fall 2004 data were combined as one season, and the spring 2003 and spring 2004 periods were kept independent from one
another. This divided all of the data into three seasonal categories, which were used to control for season.

Visible Mold and Air Concentration
Categorical rankings of visible mold were examined to determine if there were any relationships to airborne fungal spore concentrations. It was found that visible mold did not influence airborne fungal spore concentrations when analyzed with the total concentrations or the individual mold genera.

Health Outcomes
Rhinitis
No significant associations for allergic rhinitis or atopic rhinitis were found when analyzed with the total concentration. When statistical analyses were conducted on the various mold taxa, several associations were observed. An association between Basidiospore concentrations and the reporting of rhinitis (any rhinitis, regardless of whether that rhinitis was allergic or atopic) was observed after controlling for season (p < 0.01) (Figure 1). However, season was found to affect the outcome of any rhinitis and Basidiospore concentrations. It was determined that the fall season was driving this difference (geometric mean (GM) Rhinitis(-) = 23 spores/m³, GM Rhinitis(+) = 71 spores/m³). In addition, Ganoderma sp. was found to have a negative borderline relationship with rhinitis (p = 0.06; Table 3).

Allergic Sensitization
When analyzing the total concentrations with allergic sensitization to any allergen, no differences were observed between SPT(+) and SPT(-) children. However, when statistical analysis was conducted on the various mold genera, much like the rhinitis data, several
associations were observed. When determining if there were associations between children who had a positive SPT to any allergen and the various mold taxa, it was discovered that *Penicillium/Aspergillus* type spores had a positive association with SPT(+) (p < 0.01) (Figure 2). Conversely, it was determined that *Cladosporium* sp. had an inverse association with SPT(+) to any allergen (p < 0.05) (Figure 3). Additionally, *Alternaria* sp. was positively associated with SPT(+) to any allergen (p = 0.01) (Table 3).

Significant relationships were also found when examining the data for SPT to aeroallergens. A similar inverse relationship between *Cladosporium* sp. and SPT(+) to aeroallergens was observed (p = 0.03) (Figure 4) as that relationship between *Cladosporium* sp. and SPT(+) to any allergen. Furthermore, *Ganoderma* (p = 0.07) (Table 3) showed borderline significant influence to SPT(+) to aeroallergens.

SPT was also conducted for mold. However, no significance was observed between SPT(+) to mold and either total concentrations or concentrations of specific mold genera. This is likely due to the small number of children that had SPT(+) to mold. Only 16% of the study’s subjects had a positive SPT to mold (n = 24). Other borderline associations were observed between fungal spore concentrations and health outcomes (Table 3).
Discussion

This study did not indicate relationships between total fungal spore concentrations and visible mold classifications. This outcome is not necessarily a surprising one since fungal spores from visual growth are not released into the air continuously. The poor association between visible mold and air concentrations may also be due to the behavior of larger mold spores (such as Alternaria sp., Epicoccum sp., and Pithomyces sp.) that settle out of the air more quickly than smaller spores. Additionally, the area in which the visible mold was identified was not necessarily in the same room in which the air sampling took place.

We did not find associations between total fungal spore concentrations and health outcomes with the exception of total concentration and a relationship with number of children diagnosed as having rhinitis at the 10% level. When examining the various mold genera that were detected within the samples, associations between health outcomes and concentrations became apparent. Cladosporium sp., Basidiospores, Penicillium/Aspergillus type spores, and Alternaria were most frequently associated with significant health outcomes. Data indicated that exposure to certain types of fungi may increase childhood sensitization to allergens other than fungi.

Perhaps the most interesting effects observed in this study were the apparent inverse (and potentially beneficial) associations that Cladosporium and Basidiospores had on SPT(+) data. This inverse relationship may actually be explained by a potential mechanism that has been proposed for bacterial endotoxin exposures (Eduard et al., 2004). In the late 1990s, the possibility of a beneficial role of bacterial endotoxin in protecting against atopic disease was first considered (Michael, 1996). The mechanism lies in the Th1/Th2 immune paradigm that has been proposed in previous studies (Niven, 2003; Yazdanbakhsh, 2002). It is known that
microbial exposure to such things as endotoxin can induce airway inflammation and non-atopic asthma. It has been suggested that these same agents might also inhibit IgE production by inhibiting the Th2 immune responses. Although it has not been investigated extensively, fungal exposures may result in an alternative immunologic event that underlies the lack of allergy and sensitization in children (Yazdankabsh et al., 2002). The interaction between these pathways is not well understood at this time, including whether both pathways are activated by the same concentrations of microbial agents (Eduard et al., 2004). However, it is not an entirely new concept that suggests that persistent immune challenge may provide an explanation for the observed inverse association of fungal spores and health outcomes (Yazdanbakhsh et al., 2002).

A potential criticism of this study may be that previous studies have clearly identified Cladosporium sp. as a contributor to individual sensitization and to symptoms in already sensitized populations (Hasnain et al., 2002). However, studies where these findings have been indicated have been conducted in adult populations only. Very little is known about the health effects of fungal exposure during infancy. To our knowledge, this study is the first one to report an inverse association between mold exposure and health outcome (rhinitis and allergic sensitization) in infants. Eduard et al. (2004) reported similar findings for fungal exposure and atopic asthma in adults.

It is believed that contrasting relationships among the various mold genera to the health outcomes investigated in this study might actually mask the effect that total concentration may have on these outcomes. This would help to explain some of the lack of association in the reporting of health effects and total fungal spore concentrations in previous studies. However, it should be noted that the indoor environment (specifically the residential indoor environment) is a complicated one, where allergens, pollutants, and toxins coexist, and can have potential for
synergistic relationships (Niven, 2003) which were not examined in this study. Based on the
data presented in this study, it appears that clinicians and researchers should pay more attention
to the composition of the fungal spore profile and the respective concentrations of the mold
genera present rather than total or culturable spore count alone. Furthermore, this study was
carried out in an infant population, and additional investigation in adult and occupational
populations should be conducted to determine if age is a factor in the role that fungi play in
immune response.
References


Martinez, F.D., (1999). Maturation of immune responses at the beginning of asthma. *Journal of Allergy and Clinical Immunology, 103*, 355 – 361.


Figure 1: Association Between Basidiospore Concentrations and Rhinitis (Any rhinitis). Each bar represents the geometric mean of spore concentration and error bars represent the 95% confidence interval.  

Figure 2: Association Between *Penicillium/Aspergillus* type spores and SPT(+) to any allergen.
Figure 3: Inverse Association Between *Cladosporium* sp. and SPT(+) to any allergen

Figure 4: Inverse Association Between *Cladosporium* sp. and SPT(+) to aeroallergens. SPT(-) aero represents a negative SPT to aeroallergens; SPT(+) aero represents a positive SPT to aeroallergens.
Table 1: Frequency of Mold Genera (% of homes)

<table>
<thead>
<tr>
<th></th>
<th>Indoor All Data</th>
<th>Indoor (First Sample in Each Home)</th>
<th>Outdoor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cladosporium</td>
<td>90%</td>
<td>85%</td>
<td>99%</td>
</tr>
<tr>
<td>Penicillium/ Aspergillus</td>
<td>84%</td>
<td>90%</td>
<td>100%</td>
</tr>
<tr>
<td>Basidiospores</td>
<td>77%</td>
<td>74%</td>
<td>91%</td>
</tr>
<tr>
<td>Ascospores</td>
<td>73%</td>
<td>72%</td>
<td>99%</td>
</tr>
<tr>
<td>Smuts/Myxomycetes</td>
<td>52%</td>
<td>59%</td>
<td>80%</td>
</tr>
<tr>
<td>Unknown</td>
<td>46%</td>
<td>62%</td>
<td>91%</td>
</tr>
<tr>
<td>Ganoderma</td>
<td>34%</td>
<td>23%</td>
<td>47%</td>
</tr>
<tr>
<td>Alternaria</td>
<td>24%</td>
<td>27%</td>
<td>59%</td>
</tr>
<tr>
<td>Pithomyces</td>
<td>21%</td>
<td>17%</td>
<td>25%</td>
</tr>
<tr>
<td>Epicoccum</td>
<td>19%</td>
<td>20%</td>
<td>59%</td>
</tr>
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</table>
Table 2: Geometric Mean (GM) of Total Concentration and Four Most Frequent Mold Genera by Season

<table>
<thead>
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<th>Spring '04</th>
<th>Fall '03/04</th>
</tr>
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<td></td>
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<td>Range</td>
<td>GM</td>
<td>Range</td>
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<tr>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Total Count</td>
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<td>2292</td>
<td>277</td>
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<tr>
<td>Cladosporium sp.</td>
<td>30</td>
<td>932</td>
<td>35</td>
<td>333</td>
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<tr>
<td>Penicillium/Aspergillus</td>
<td>24</td>
<td>1205</td>
<td>161</td>
<td>602</td>
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<tr>
<td>Basidiospores</td>
<td>17</td>
<td>1091</td>
<td>16</td>
<td>133</td>
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<tr>
<td>Ascospores</td>
<td>8</td>
<td>343</td>
<td>16</td>
<td>116</td>
</tr>
<tr>
<td><strong>Outdoor</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Count</td>
<td>2469</td>
<td>9980</td>
<td>2474</td>
<td>4610</td>
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<td>Cladosporium sp.</td>
<td>365</td>
<td>6564</td>
<td>321</td>
<td>2067</td>
</tr>
<tr>
<td>Penicillium/Aspergillus</td>
<td>1144</td>
<td>3281</td>
<td>1258</td>
<td>2247</td>
</tr>
<tr>
<td>Basidiospores</td>
<td>113</td>
<td>1188</td>
<td>114</td>
<td>966</td>
</tr>
<tr>
<td>Ascospores</td>
<td>202</td>
<td>1424</td>
<td>492</td>
<td>1201</td>
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</table>

Table 3: Selected Results of Associations Among SPT and Rhinitis and Fungal Spore Concentrations

<table>
<thead>
<tr>
<th>Genus</th>
<th>P-values (Positive (+) or Negative (-) Association)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Outcome/Atopy</td>
</tr>
<tr>
<td></td>
<td>SPT</td>
</tr>
<tr>
<td>Total Concentration</td>
<td>NS³</td>
</tr>
<tr>
<td>Alternaria sp.²</td>
<td>0.01 (+)</td>
</tr>
<tr>
<td>Ganoderma²</td>
<td>0.10 (-)</td>
</tr>
<tr>
<td>Pithomyces sp.²</td>
<td>NS</td>
</tr>
<tr>
<td>Basidiospores³</td>
<td>0.09 (-)</td>
</tr>
<tr>
<td>Penicillium/Aspergillus type³</td>
<td>0.01 (+)</td>
</tr>
<tr>
<td>Cladosporium sp.³</td>
<td>0.04 (-)</td>
</tr>
</tbody>
</table>

¹ P-values ≤ 0.10 are reported only
² Classified as <LOD or ≥ LOD and analyzed by c² testing
³ Modeled continuously and analyzed by logistic regression
⁴ NS = Not significant
Appendix A

Frequency Distributions for Fungal Spore Concentrations by Season, Total Concentration, and Mold Genera
Appendix B

SPSS Data: Descriptive Stats for 3 Season Categories, Total Concentration and Four Most Frequent Mold Genera\(^1,2\)

Descriptive Stats for 3 Season Categories and Total Concentrations

<table>
<thead>
<tr>
<th>threeseason</th>
<th>N</th>
<th>Mean</th>
<th>Std. Error of Mean</th>
<th>Range</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Geometric Mean</th>
<th>Std. Deviation</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26</td>
<td>383.5300</td>
<td>56.30118</td>
<td>1241.81</td>
<td>10.05</td>
<td>1251.86</td>
<td>276.6209</td>
<td>287.08081</td>
<td>82415.390</td>
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<tr>
<td>2</td>
<td>75</td>
<td>97.5779</td>
<td>13.43196</td>
<td>565.14</td>
<td>2.94</td>
<td>568.08</td>
<td>53.9450</td>
<td>116.32417</td>
<td>13531.312</td>
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<td>3</td>
<td>180</td>
<td>404.2518</td>
<td>32.74391</td>
<td>2284.72</td>
<td>9.80</td>
<td>2294.52</td>
<td>229.1785</td>
<td>439.30567</td>
<td>192989.5</td>
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<tr>
<td>Total</td>
<td>281</td>
<td>320.4820</td>
<td>23.29596</td>
<td>2291.58</td>
<td>2.94</td>
<td>2294.52</td>
<td>158.5109</td>
<td>390.51141</td>
<td>152499.2</td>
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</table>

Descriptive Stats for 3 Season Categories and Penicillium/Aspergillus type Spore Concentrations

<table>
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<th>threeseason</th>
<th>N</th>
<th>Mean</th>
<th>Std. Error of Mean</th>
<th>Range</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Geometric Mean</th>
<th>Std. Deviation</th>
<th>Variance</th>
</tr>
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<tbody>
<tr>
<td>1</td>
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<td>219.7846</td>
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<td>11.05</td>
<td>612.65</td>
<td>160.5481</td>
<td>159.32128</td>
<td>25383.269</td>
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<td>2</td>
<td>75</td>
<td>58.6613</td>
<td>8.25347</td>
<td>391.55</td>
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<td>392.55</td>
<td>31.3153</td>
<td>71.47717</td>
<td>5108.985</td>
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<td>181</td>
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<td>1.00</td>
<td>1206.24</td>
<td>15.8685</td>
<td>182.65309</td>
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<tr>
<td>Total</td>
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<td>9.75013</td>
<td>1205.24</td>
<td>1.00</td>
<td>1206.24</td>
<td>23.5352</td>
<td>163.73253</td>
<td>26808.342</td>
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</table>

\(^1\) Season 1 = Spring '03; Season 2 = Spring '04; Season 3 = Fall '03 and '04
\(^2\) A value of 1 must be subtracted from all data in order to correct for the adjustment made for data that was below the LOD.
### Descriptive Stats for 3 Season Categories and Ascospore Concentrations

**ascomod**

<table>
<thead>
<tr>
<th>threeseason</th>
<th>N</th>
<th>Mean</th>
<th>Std. Error of Mean</th>
<th>Range</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Geometric Mean</th>
<th>Std. Deviation</th>
<th>Variance</th>
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<tbody>
<tr>
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<td>25.9804</td>
<td>4.73931</td>
<td>115.69</td>
<td>1.00</td>
<td>116.69</td>
<td>15.5234</td>
<td>24.16581</td>
<td>583.987</td>
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<tr>
<td>2</td>
<td>75</td>
<td>6.4104</td>
<td>1.01316</td>
<td>44.94</td>
<td>1.00</td>
<td>45.94</td>
<td>3.2672</td>
<td>8.77423</td>
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<td>181</td>
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<td>344.35</td>
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<tr>
<td>Total</td>
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<td>21.7996</td>
<td>2.25994</td>
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<td>344.35</td>
<td>7.9015</td>
<td>37.95090</td>
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</table>

### Descriptive Stats for 3 Season Categories and Basidiospore Concentrations

**basimod**

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<th>Mean</th>
<th>Std. Error of Mean</th>
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<th>Maximum</th>
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<th>Std. Deviation</th>
<th>Variance</th>
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<tbody>
<tr>
<td>1</td>
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<td>28.5369</td>
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<td>2</td>
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<td>7.6961</td>
<td>1.83673</td>
<td>95.49</td>
<td>1.00</td>
<td>96.49</td>
<td>2.6489</td>
<td>15.90657</td>
<td>253.019</td>
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<td>181</td>
<td>128.2051</td>
<td>15.29538</td>
<td>1090.65</td>
<td>1.00</td>
<td>1091.65</td>
<td>36.5054</td>
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<td>Total</td>
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<td>86.9656</td>
<td>10.37847</td>
<td>1090.65</td>
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<td>1091.65</td>
<td>16.8718</td>
<td>174.28422</td>
<td>30374.990</td>
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</table>

1 Season 1 = Spring '03; Season 2 = Spring '04; Season 3 = Fall '03 and '04
2 A value of 1 must be subtracted from all data in order to correct for the adjustment made for data that was below the LOD.
### Descriptive Stats for 3 Season Categories and Cladosporium sp. Concentrations

<table>
<thead>
<tr>
<th>threeseason</th>
<th>N</th>
<th>Mean</th>
<th>Std. Error of Mean</th>
<th>Range</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Geometric Mean</th>
<th>Std. Deviation</th>
<th>Variance</th>
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</thead>
<tbody>
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</tr>
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1. Season 1 = Spring '03; Season 2 = Spring '04; Season 3 = Fall '03 and '04.
2. A value of 1 must be subtracted from all data in order to correct for the adjustment made for data that was below the LOD.
Appendix C

SPSS Data: ANOVA – Scheffe Tests for Seasonal Differences

Post Hoc Tests - ANOVA Scheffe for Seasonal Differences

<table>
<thead>
<tr>
<th>(I) Sample Date with Year</th>
<th>(J) Sample Date with Year</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% Confidence Interval</th>
<th>Lower Bound</th>
<th>Upper Bound</th>
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<td>11</td>
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<td>11</td>
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<td>.000</td>
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<tr>
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<td>.21058</td>
<td>.000</td>
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</tr>
</tbody>
</table>

* The mean difference is significant at the .05 level.

1 Seasonal comparisons where: 11 = Spring ’03; 12 = Fall ’03; 21 = Spring ’04; 22 = Fall ‘04
## Appendix D

**Descriptive Statistics for Significant and Borderline Significant Outcomes**

### Case Summary for Any Rhinitis and Total Concentration

<table>
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<th>Grand Total</th>
<th>Log of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
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<td>91</td>
</tr>
<tr>
<td>Mean</td>
<td>308.1638</td>
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</tr>
<tr>
<td>Std. Error of Mean</td>
<td>39.65758</td>
<td>.15069</td>
</tr>
<tr>
<td>Minimum</td>
<td>2.94</td>
<td>1.08</td>
</tr>
<tr>
<td>Maximum</td>
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</tr>
<tr>
<td>Range</td>
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</tr>
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<td>Std. Deviation</td>
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<tr>
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<td>54</td>
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### Case Summary for SPT Any and Penicillium/Aspergillus type Concentrations

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### Case Summary for SPT Any and Basidiospore Concentrations

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1 Where category 0 is a negative outcome and category 1 is a positive outcome.
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|       | Std. Error of Mean | 10.77127 | .19543   |
|       | Minimum  | 1.00     | .00      |
|       | Maximum  | 621.84   | 6.43     |
|       | Range    | 620.84   | 6.43     |
|       | Std. Deviation | 87.50617 | 1.58766  |
|       | Geometric Mean | 18.8234  | .0000    |

### Case Summary for SPT Aero and Penicillium/Aspergillus type Concentrations

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|       | Maximum | 1153.98  | 7.05     |
|       | Range   | 1152.98  | 7.05     |
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|       | Geometric Mean | 53.8491  | .0000    |

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1 Where category 0 is a negative outcome and category 1 is a positive outcome.
### Case Summary for SPT Aero and Cladosporium Concentrations

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### Case Summary for SPT Any and Alternaria Concentrations

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1 Where category 0 is a negative outcome and category 1 is a positive outcome
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Case Summary for SPT Aero and Ganoderma Concentrations

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1 Where category 0 is a negative outcome and category 1 is a positive outcome
### Case Summary for Any Rhinitis and Alternaria Concentrations

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<td>Std. Deviation</td>
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| 1 N         | 54     | 54       |
| Mean        | 3.2376 | .4485    |
| Median      | 1.0000 | .0000    |
| Std. Error of Mean | .89536 | .12766 |
| Minimum     | 1.00   | .00      |
| Maximum     | 39.28  | 3.67     |
| Range       | 38.28  | 3.67     |
| Geometric Mean | 1.5660 | .0000    |
| Std. Deviation | 6.57956 | .93810 |

| Total N     | 145    | 145      |
| Mean        | 3.7079 | .6063    |
| Median      | 1.0000 | .0000    |
| Std. Error of Mean | .51374 | .08388 |
| Minimum     | 1.00   | .00      |
| Maximum     | 39.28  | 3.67     |
| Range       | 38.28  | 3.67     |
| Geometric Mean | 1.8336 | .0000    |
| Std. Deviation | 6.18626 | 1.01008 |

### Case Summary for Any Rhinitis and Ganoderma

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| 1 N         | 54     | 54       |
| Mean        | 13.4900 | 1.0133  |
| Median      | 1.0000 | .0000    |
| Std. Error of Mean | 3.90330 | .22103 |
| Minimum     | 1.00   | .00      |
| Maximum     | 129.96 | 4.87     |
| Range       | 128.96 | 4.87     |
| Geometric Mean | 2.7548 | .0000    |
| Std. Deviation | 28.68325 | 1.62422 |

| Total N     | 145    | 145      |
| Mean        | 7.4699 | .6507    |
| Median      | 1.0000 | .0000    |
| Std. Error of Mean | 1.62105 | .10813 |
| Minimum     | 1.00   | .00      |
| Maximum     | 129.96 | 4.87     |
| Range       | 128.96 | 4.87     |
| Geometric Mean | 1.9170 | .0000    |
| Std. Deviation | 19.51999 | 1.30204 |

1 Where category 0 is a negative outcome and category 1 is a positive outcome
## Case Summary for Atopic Rhinitis and Pithomyces

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<td>Std. Deviation</td>
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1 Where category 0 is a negative outcome and category 1 is a positive outcome
Appendix E

Univariate GLM - Significant and Borderline Significant Associations for Health Outcomes, Total Concentrations and Four Most Frequent Mold Genera: Controlled for Season

Any Rhinitis and Total Concentration Controlled for Season

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<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
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<td>89.423</td>
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ᵃ. R Squared = .324 (Adjusted R Squared = .310)
Association of Any Rhinitis and Basidiospores Controlled for Season

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<td>101.202</td>
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<sup>a</sup> R Squared = .386 (Adjusted R Squared = .373)

Association of SPT Any and Penicillium/Aspergillus type Controlled for Season

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<sup>a</sup> R Squared = .163 (Adjusted R Squared = .145)
### Association of SPT Any and Basidiospores Controlled for Season

**Dependent Variable: baslnmod**

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\(^a\) R Squared = .366 (Adjusted R Squared = .352)

### Association of SPT Any and Cladosporium Controlled for Season

**Dependent Variable: clainmod**

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<th>Sig.</th>
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\(^a\) R Squared = .273 (Adjusted R Squared = .257)
Association of SPT Aero and Penicillium/Aspergillus Controlled for Season

Dependent Variable: penlnmod

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<tr>
<td>Corrected Total</td>
<td>452.930</td>
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a. R Squared = .136 (Adjusted R Squared = .117)

Association of SPT Aero and Cladosporium Controlled for Season

Dependent Variable: clnlnmod

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
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<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
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a. R Squared = .274 (Adjusted R Squared = .258)
Appendix F

Crosstabular Chi Square - Significant and Borderline Significant Associations Between Health Outcomes and Less Frequent Mold Genera

Association Between SPT Any and Alternaria

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
<th>df</th>
<th>Asymp. Sig. (2-sided)</th>
<th>Exact Sig. (2-sided)</th>
<th>Exact Sig. (1-sided)</th>
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<tbody>
<tr>
<td>Pearson Chi-Square</td>
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<td>Linear-by-Linear</td>
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</table>

a. Computed only for a 2x2 table
b. 0 cells (.0%) have expected count less than 5. The minimum expected count is 20.
   17.
### Association Between SPT Any and Ganoderma

<table>
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<td>N of Valid Cases</td>
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</tbody>
</table>

\(^a\) Computed only for a 2x2 table

\(^b\) 0 cells (.0%) have expected count less than 5. The minimum expected count is 15.

### Association Between SPT Aero and Ganoderma

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<th>Exact Sig. (2-sided)</th>
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</tbody>
</table>

\(^a\) Computed only for a 2x2 table

\(^b\) 0 cells (.0%) have expected count less than 5. The minimum expected count is 12.

38.
### Association Between Any Rhinitis and Alternaria

<table>
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<th>Exact Sig. (2-sided)</th>
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</table>

a. Computed only for a 2x2 table  
b. 0 cells (.0%) have expected count less than 5. The minimum expected count is 16, 39.

### Association Between Any Rhinitis and Ganoderma

<table>
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a. Computed only for a 2x2 table  
b. 0 cells (.0%) have expected count less than 5. The minimum expected count is 12, 29.
### Association Between Atopic Rhinitis and Pithomyces

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a. Computed only for a 2x2 table  

b. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 4.  

73.