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Molds, mycotoxins, and sick building syndrome

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Abstract

The following is a review of some of the work we have done since 2004 regarding the importance of molds and their mycotoxins in the phenomenon of sick building syndrome (SBS). In these studies we showed that the macrocyclic trichothecene mycotoxins (MTM) of Stachybotrys chartarum (SC) are easily dissociated from the surface of the organism as it grows and could therefore be consequently spread in buildings as the fungus experiences additional water events. We then showed that SC and Penicillium chrysogenum (PC) colonies remain viable long after a water source has been removed, and the MTM produced by SC remain toxic over extended periods of time. We next showed that PC when inhaled, can release in vivo, a protease allergen that can cause a significant allergic inflammatory reaction in the lungs of mice. We then showed, in a laboratory study, that the MTM of SC can become airborne attached to spores or SC particulates smaller than spores. Following that study, we next showed that the same phenomenon actually occurred in SC infested buildings where people were complaining of health problems potentially associated with SBS. Finally, we were able to demonstrate the presence of MTM in the sera of individuals who had been exposed to SC in indoor environments. This last study was done with enough mold exposed individuals to allow for the statistical significance of SC exposure to be evaluated.

Keywords

Molds, mycotoxins, sick building syndrome, *Stachybotrys chartarum*, *Penicillium chrysogenum*

Introduction

'Yeasts, molds, mushrooms, mildews, and the other fungi pervade our world. They work great good and terrible evil. Upon them, indeed, hangs the balance of life; for without their presence in the cycle of decay and regeneration, neither man nor any other living thing could survive' (Kavaler, 1965).

I used the above paragraph to introduce our book chapter entitled 'Fungi and the Indoor Environment: Their Impact on Human Health.' (Cooley et al., 2004). It was in a book I edited in 2004 entitled 'Sick Building Syndrome.' That chapter detailed the work we had done on sick building syndrome (SBS) from 1998 to 2002. What I would like to do in this review is to describe the work that has been done in my laboratory since that chapter was written to the present day. I intend for this review to be a continuation of the chapter published in 2004. For those of you good readers who are not familiar with the 2004 chapter, I will briefly reiterate what we published back then with the idea of setting the stage for the present (2009) review.

The following are some of the things that we now know about the role of fungi in SBS established prior to the writing of this review. First of all, it is now known that fungal infestation of buildings has plagued man from the time of Moses, over 3300 years ago (Leviticus). The people of Moses' time knew that mold growth in a building was problematic, but what they did not know was 'why' this was so. Today, we know what these organisms are and what the products they produce are with the potential to cause human health problems.

Our first published article on SBS appeared in 1998 and it showed that there was a correlation between the presence of certain fungi in buildings and this

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syndrome. These fungi were *Penicillium* species and *Stachybotrys chartarum* (SC; Cooley et al., 1998). In this study, we hypothesized that it was the inhalation of high concentrations of *Penicillium* species spores from the air and the inhalation of SC mycotoxins from the air that had the potential to effect human health.

We next developed an animal model for allergic penicilliosis in an effort to try to determine why the inhalation of *Penicillium* species spores could produce human health problems. The animal model we used employed the intranasal (IN) instillation of 10^4 viable (V) and non-viable (NV) *Penicillium chrysogenum* (PC) conidia into mice (Cooley et al., 2000). Therefore, we could assume that over the period of the 6 weeks of exposure, if there were any differences between the two groups of C57 black/6 mice, they could be attributed to the viability of the PC. Results showed that the mice receiving the V PC produced significantly more total serum immunoglobulin E (IgE), airway eosinophils and peripheral eosinophils than did those mice receiving the NV PC. This article demonstrated that 6 weeks of inhalation of V PC by C57 black/6 mice caused a type 2 T helper cell-directed inflammatory response.

We then examined the cellular and humoral response of C57 black/6 mice following the IN instillation of V PC spores (Cooley et al., 1999). In this research, we found that V PC spores were recoverable from rodent lungs, as quickly as one quarter of an hour and from 3 hours up to 36 hours after IN instillation of 10^6 V spores. We also found that 10^6 doses of V PC spores induced statistically significant increases in tumor necrosis factor-α while the NV spores of PC did not have the same capability. These studies led us to surmise that V PC spores might be capable of producing antigenic material in vivo, because they could survive inside the lungs for at least 36 hours. Indeed, we were able to show that living PC spores are capable of the production of such material (Schwab et al., 2003). In this study, we showed that mice did not develop allergic reactions to challenge by low levels (10^2) of V PC conidia. The antigens that we isolated from V PC conidia were protease allergens that did induce specific allergic responses in mice. This indicated the importance of PC spores in allergic sensitization to this fungus.

In a 1999 study (McGrath et al.), we showed that when indoor air (IDA) samples for fungal profile are taken, they accurately reflect the air in that building for at least 6 hours. That is to say, IDA conditions do not fluctuate wildly over short periods of time. In this study, we also demonstrated that outdoor air (ODA) fungal profile samples fluctuated dramatically over the 6-hour period we examined. This was expected because the prevailing winds are continually blowing new spores into an area and blowing old spores away from the same locale. In this same study, we also showed that 'sick' buildings stay contaminated over extended periods of time and they do not get 'better' and then become contaminated again. Once a building is contaminated with mold, it stays that way until it is remediated.

We next decided to see if it was possible to develop building material that would not allow mold growth, even if it was wetted. We decided to examine this because it is literally impossible to keep water out of buildings. This is because roofs will always leak, pipes will always break and water events (such as floods) will always occur. In this study (Karunasena et al., 2000), we examined the growth of SC, PC, and *Cladosporium cladosporioides* on organic ceiling title (OCT) and inorganic ceiling tile (ICT). Both OCT and ICT were wetted and inoculated with the above three fungal genera. While all the three organisms grew on the OCT, none grew on the ICT, even when an organic food source such as tryptic soy broth was added. Therefore, the development of mold-resistant building material is possible and should be pursued.

The last study I included in the 2004 chapter (Cooley et al., 2004) examined something that no one had thought of looking at before. That is, the relationship between SBS-associated fungi and the reality that it is difficult to get zoo animals to breed in captivity. The obvious question was, could extended exposure to SBS-associated molds, affect animal morbidity and/or mortality and then ultimately breeding success? We examined five zoos across the United States and a total of 110 sites from those zoos for the presence or absence of SBS-associated fungi, as well as whether there was a correlation between the presence of these organisms and animal morbidity (Wilson and Straus, 2002). We examined surfaces for fungal growth and the air for evidence of fungal proliferation. This research showed that high levels of PC spores were found in the air of all five zoos. Also SC growth sites were found in two of the zoos. This is not surprising because water hoses are used indiscriminately in zoos to clean animal cages and exhibits, so it is not unusual to see cellulose-containing building materials that stay wet for long periods of time. This, of course, leads to mold growth. A large number of fungal species, aside from PC and SC,

were found at all zoos. In this study, we showed a non-random, significant (Fisher exact test analysis, $p < .001$) relationship existed between zoos with high levels of airborne PC and documented animal morbidity.

Finally, the last thing I discussed in the 2004 chapter on the effects of fungi on human health was the role of mycotoxins in SBS. Mycotoxins are secondary metabolites produced by fungi as they grow. A secondary metabolite is something an organism produces while it grows, but is not necessary for its survival. I left the question open as to whether or not mycotoxins play a role in SBS. We have made a great deal of progress in answering this question in the last 4 years, but one important question remains to be answered. Before I tell the reader what that question is, I would like to tell you what we do know for sure. We know that mycotoxins are produced in buildings when fungi grow on building materials inside them (Croft et al., 1986; Engelhard et al., 2002; Nielson et al., 1999; Nieminen et al., 2002; Nikulin et al., 1994; Tuomi et al., 2000). We know that macrocyclic trichothecene mycotoxins (MTM) exist on the conidia of the SC spores (Gregory et al., 2004; Sorenson et al., 1987) and that these spores can be inhaled by humans (Elidemir et al., 1999). We know that these MTM definitely get into the air in SC-contaminated buildings where they can be inhaled (Brasel et al., 2005b). We know that the MTM are definitely inhaled by people in these buildings (Brasel et al., 2004; Yike et al., 2006). The final question that remains to be answered is this: do the MTM of SC get into humans in sufficient concentration to cause human disease? We do not, as of yet, know the answer to that question, but in the following pages, I will supply additional information in pursuit of this question and many others.

Protein translation inhibition by SC conidia with and without the mycotoxin containing polysaccharide matrix

One of the most perplexing questions regarding SC presence in a building was the following: if SC spores are not commonly found in the air in these buildings, why do individuals in these structures complain of symptoms that could be attributed to exposure to SC MTM? This is the question that we attempted to answer with our 2004 study (Karunasena et al., 2004). We thought that perhaps there would be a simple explanation for SC MTM to be found in buildings in the absence of SC spores. It was well known that

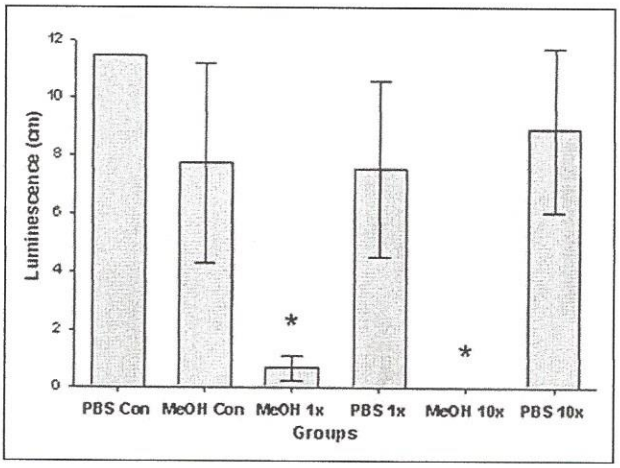

Figure 1. Protein synthesis inhibition by *Stachybotrys* spores washed 10 times with PBS solution or methanol. Spores washed once with phosphate buffered saline (PBS; 1×) or methanol (MeOH; 1×) and spores washed 10 times with PBS (10×) or MeOH (10×) were incubated with rabbit reticulocyte lystate system. Luminescence was measured in centimeters (cm). The single asterisk (*) indicates a significant difference ($p < .05$) in luminescence (cm) production by spores washed with methanol once or 10 times compared to the PBS (PBS Con) and methanol (MeOH Con) controls. The error bars represent standard deviation from the mean. Luminescence was measured in centimeters (cm). An all-pairwise multiple comparison procedure (Dunn's Method was used ($p < .05$) to compare the spores washed with PBS and methanol. Reproduced with permission from Karunasena et al., 2004.

SC colonies produce a polysaccharide matrix, which surrounds the colony (Karunasena et al., 2004). It is thought that the SC colony produces this carbohydrate 'capsule' in order to prevent its water from evaporating. Whatever the reason for the presence of this carbohydrate matrix, we thought that the MTM might be found in this carbohydrate matrix (CM) and if this CM separated from the SC colony, it would supply a source of inhalable MTM. Therefore, we determined whether the CM and the MTM embedded in it could be removed from the SC spores by either organic or aqueous solvents. In order to do this, we used a protein translation inhibition assay (Yike et al., 1999), because it is well known that the MTM of SC inhibit protein synthesis.

The obtained results showed that the process of conidial aqueous washing removed material that was capable of inhibiting protein synthesis as compared to spores that were washed in an organic solution (Figures 1 and 2). We also found no correlation between CM removal during conidial washing and the

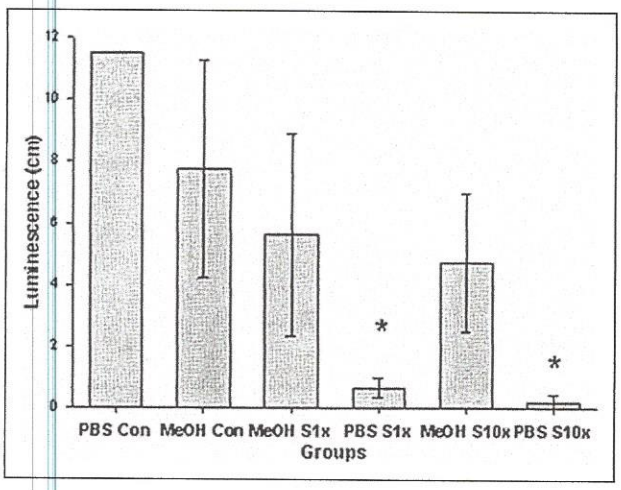

Figure 2. Protein synthesis inhibition of supernatants of *Stachybotrys* spores washed once or 10 times with PBS or methanol. The single asterisk (*) indicates a significant difference ($p < .05$) by the supernatant of spores washed with PBS once (PBS S1×) or 10 times (phosphate buffered saline [PBS] S10×) when compared to the PBS control (PBS Con), or methanol control (MeOH Con). The error bars represent standard deviation from the mean. An all-pairwise multiple comparison procedure (Dunn's Method) was used ($p < .05$) to compare the supernatants of spores washed with PBS and methanol. Reproduced with permission from Karunasena et al., 2004.

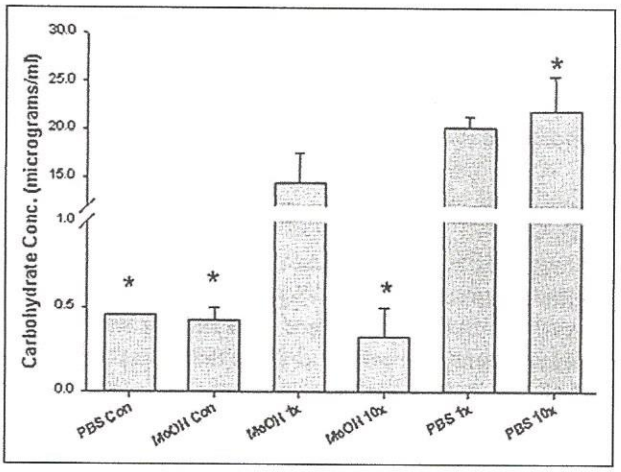

Figure 3. Carbohydrate concentration of supernatants from *Stachybotrys* spores washed with phosphate buffered saline (PBS) or methanol. An anthrone assay was conducted to identify the concentration of carbohydrate obtained from the supernatant of spores washed once (PBS 1×), (MeOH 1×), and 10 times (PBS 10×), (MeOH 10×) with PBS or methanol and potato dextrose agar (PDA) media washed with PBS (Media PBS) or methanol (Media MeOH). The single asterisk (*) indicates a significant difference ($p < .05$) in the amount of carbohydrate in the supernatant of spores washed with PBS when compared to the PBS control (PBS Con), methanol control (MeOH Con), or MeOH supernatant from spores washed 10 times (MeOH S10×). An all-pairwise multiple comparison procedure (Dunn's Method) was used ($p < .05$) to compare the supernatants of the PBS and methanol washed spores. Reproduced with permission from Karunasena et al., 2004.

removal of MTM from the spore surface (Figure 3). These data showed that MTM are not found exclusively in the CM of SC conidia, and that MTM removal from the spore surface can occur without demonstrable loss of CM. We also demonstrated that MTM can be removed from the surface of SC conidia by aqueous solution. This is of considerable importance because the MTM found on the surface of SC colonies inside buildings could be distributed to other parts of the building during a 'water event' due to their solubility in water. Then when the water dries up, and the MTM are left in the building dust, air currents can pick up these compounds and distribute them throughout the building where they could be inhaled by the inhabitants. This theory was proposed by other investigators as well. Hinkley and Jarvis stated in 2001, 'Such aqueous solubility of the toxins may further their distribution in the natural environment and increase their human health risk.'

Culturability and toxicity of SBS related fungi over time

In this study, two experiments were conducted to examine the culturability and toxicity of various molds placed on building material over time as well as the efficiency of seven laboratory procedures designed to recover viable fungi from swab samples (Wilson et al., 2004). In the first study, SC was inoculated onto eight different pieces of drywall and kept at room temperature (RT) and 20%- 60% relative humidity (RH) for as long as 2 years. Eight additional pieces of ceiling tile (CT) were kept at 100% RH for 365 days. Also, 6 pieces of CT and 15 cotton swabs (CS) were inoculated with PC and SC, respectively, and kept under similar conditions for 240 days and 3 and one-third years. At the end of the specified time period, all materials were tested for fungal viability. Results from this study demonstrated that all samples were culturable after the specified storage period and that the drywall inoculated with SC still tested positive for MTM (Table 1 and Figures 4 and 5). In the second part of this study, SC and *Chaetomium globosum* (CG) were inoculated onto 84 CS each. The CS were then stored up to 266 days at RT and

Table 1. Results from an experiment that examined the toxicity and culturability of SBS-related organisms kept in building materials at varying intervals of storage under different environmental conditions[a]

	N	% RH	Temperature (°C)	Storage time	Organism	Culturable after storage	Toxic after storage
Drywall	7	20–60	25	1 year	*S. chartarum*	Yes	Yes
Drywall	1	20–60	25	2 years	*S. chartarum*	Yes	Yes
Ceiling tile	8	100	25	1 year	*S. chartarum*	Yes	Yes
Ceiling tile	6	20–60	25	8 months	*P. chrysogenum*	Yes	Not tested
Swabs	15	20–60	25	3.3 years	*S. chartarum*	Yes	Unclear

RH, relative humidity SBS, sick building syndrome.
[a] Reproduced with permission from Wilson et al., 2004 (www.informaworld.com)

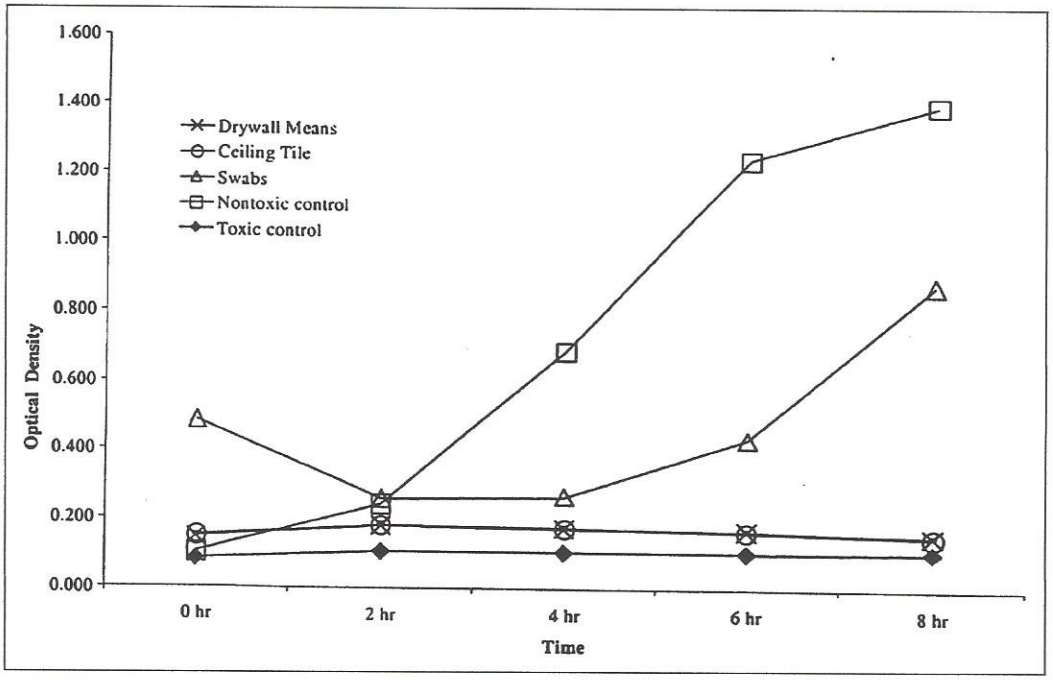

Figure 4. Results from experiment 1 that examined the toxicity of swabs, drywall and ceiling tile inoculated with *S. chartarum* after a prolonged period of storage. Toxicity analyzed using a yeast toxicity assay whereby an increase in optical density over time indicates non-toxicity. Toxic and non-toxic controls included for comparison purposes. Standard errors for drywall and ceiling tiles are all less than 0.011 optical density. Reproduced with permission from Wilson et al., 2004 (www.informaworld.com).

20%-60% RH. Seven different techniques were compared to examine the recovery of viable fungi from the CS over various time periods. These seven different techniques were: 1) streak a dry CS on to potato dextrose agar (PDA) and malt extract agar (MEA); 2) use a wet CS (0.4 ml of phosphate buffered saline [PBS]) for 5 hours at RT, then streak onto PDA and MEA; 3) wet a CS in 0.4 ml distilled water (DW) for 5 hours at RT, then streak onto PDA and MEA; 4) wet a CS in 0.4 ml PBS plus 0.05% Tween-20 (pH 7.0) for 5 hours at RT, then streak onto PDA and MEA; 5) add 5 ml DW to a CS, wait 5 hours, and then perform serial dilutions of the sample in DW. Then plate 0.1 ml of each dilution onto PDA and MEA; 6) add 5 ml of PBS to a CS, wait 5 hours, and then perform serial dilutions in PBS. Then plate 0.1 ml of each dilution onto PDA and MEA; and finally, 7) add 5 ml of PBS to a CS plus 0.05% Tween-20, wait 5 hours, and then perform serial dilutions in PBS plus 0.05% Tween-20. Then plate 0.1 ml of each dilution on PDA and MEA. All of the above seven techniques showed high rates of fungal recovery. These two studies demonstrate that even after the removal of water following a 'water event', over extended periods of time

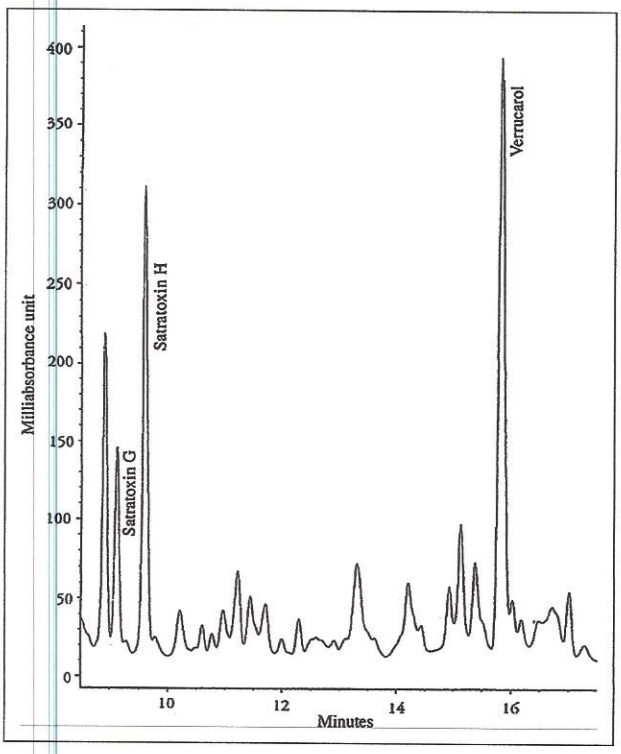

Figure 5. High performance liquid chromatography analysis of an *S. chartarum* contaminated section of drywall stored without a water source for 1 year under conditions similar to those inside human dwellings. The labeled peaks are consistent with those found for some trichothecene mycotoxins produced by *S. chartarum*. Reproduced with permission from Wilson et al., 2004 (www.informaworld.com).

and under conditions like those which would be found inside people-occupied buildings, PC and SC colonies would still be viable. Also, the MTM produced by SC are still toxic over long periods of time. Finally, in this study, we demonstrated that a wide variety of CS preparation techniques are adequate to recover SC and CG from inoculated swabs.

Allergic inflammation induced by a PC conidia-associated allergen extract in a murine model

In 2003, we characterized exposure to low levels (10^2) of V PC conidia in mice as well as allergic sensitization from conidia of the same fungus (Schwab et al., 2003). (In that article), we demonstrated that mice challenged IN with low (those levels commonly found in the ODA) levels of PC did not demonstrate allergic reaction to these organisms. We also showed that V PC spores produced a protease allergen that did induce allergic reactions in mice. This study showed

Table 2. Treatment protocol for sensitization to Pen ch protease extract

Group	Description of treatment	Dose of treatment
1	PBS/alum IP weekly for 5 weeks, PBS IN weekly for 2 weeks	200 μL PBS/alum IP, 50 μl PBS IN
2	10 μg Pen ch IP primary. 0.1 μg Pen ch IP for 4 weeks, 10 μg Pen ch IN for 2 weeks	200 μL Pen ch/alum IP, 50 μL Pen ch IN
3	10 μg Pen ch IP primary. 1.0 μg Pen ch IP for 4 weeks, 10 μg Pen ch IN for 2 weeks	200 μL Pen ch/alum IP, 50 μL Pen ch IN
4	10 μg Pen ch IP primary. 10 μg Pen ch IP for 4 weeks, 10 μg Pen ch IN for 2 weeks	200 μL Pen ch/alum IP, 50 μL Pen ch IN
5	100 μg Pen ch IP primary. 0.1 μg Pen ch IP for 4 weeks, 10 μg Pen ch IN for 2 weeks	200 μL Pen ch/alum IP, 50 μL Pen ch IN
6	100 μg Pen ch IP primary. 1.0 μg Pen ch IP for 4 weeks, 10 μg Pen ch IN for 2 weeks	200 μL Pen ch/alum IP, 50 μL Pen ch IN
7	100 μg Pen ch IP primary. 10 μg Pen ch IP for 4 weeks, 10 μg Pen ch IN for 2 weeks	200 μL Pen ch/alum IP, 50 μL Pen ch IN
8	10 μg Der p 1 IP weekly for 5 weeks, 10 μg Der p 1 IN for 2 weeks	200 μL Der p1/ alum IP, 50 μL *Der p* 1 IN

IN, intranasal; IP, intraperitoneal; PBS, phosphate-buffered saline. Reproduced with permission from Schwab et al., 2004.

the consequences of the IN inhalation of PC in allergic sensitization caused by this organism. In the present study (Schwab et al., 2004), we examined the induced allergic inflammation in mice caused by the inhalation of a PC conidium- associated allergen extract. In this work, mice were exposed to the protease from PC conidia that we had previously isolated and characterized (Schwab et al., 2003). C57black/6 mice were treated with primary intraperitoneal (IP) injections of 10 or 100 μg of the PC protease extract (Pen ch) adsorbed to alum. These injections were followed by weekly IP injections of 0.1, 1.0 or 10 μg of Pen ch with alum for 1 month, and with 10 μg of Pen ch by IN instillation for 14 days prior to sacrifice (Table 2). The results were as follows: injections of 10 and 100 μg of the protease extract for 35 days followed by 14 days of IN instillation of 10 μg of Pen ch induced significant elevations of total serum

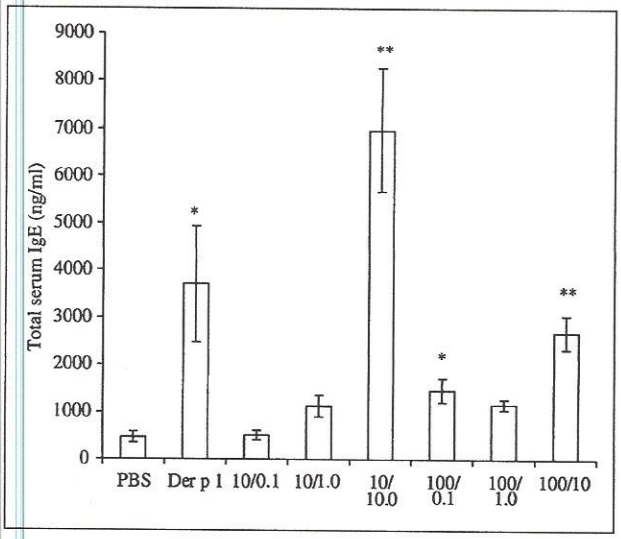

Figure 6. Serum immunoglobulin (Ig)E levels after 7 weeks of intraperitoneal and intranasal inoculations with various concentrations of Pen ch. Bars represent mean serum total IgE concentrations from each treatment group ($n = 6$). $*p < .02$ and $**p < .001$ compared with the negative control animals (phosphate-buffered saline). Error bars represent SEM. Data are representative of two independent experiments (0.1, 1.0, 10, 100 = Pen ch in μg). Reproduced with permission from Schwab et al., 2004.

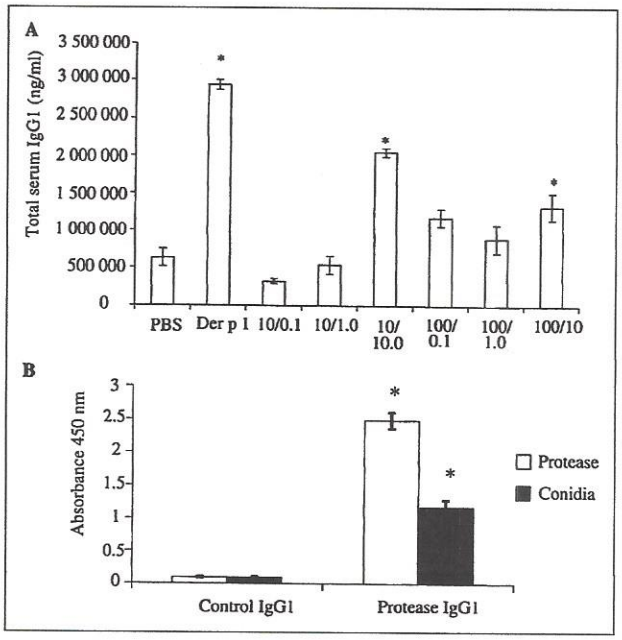

Figure 7. Total serum and Pen ch-specific immunoglobulin (Ig)G1 after 7 weeks of intraperitoneal and intranasal inoculations with various Pen ch. Data bars in (A) represent mean total serum IgG1 concentrations from each treatment group ($n = 6$). Data bars in (B) represent absorbance at 450 nm as described in materials and methods using pooled sera from mice sensitized to 10 μg of Pen ch and incubated with either 10 μg of Pen ch or 1×10^6 conidia bound to immunoplates. $*p < .01$ for sera from mice sensitized to various concentrations of Pen ch compared with sera from mice inoculated with phosphate buffered saline (PBS) only (PBS in A) and pooled sera from mice sensitized to 10 μg of Pen ch compared with sera from mice treated with PBS only (control IgG1 in B). Error bars represent SEM. Data are representative of two independent experiments (0.1, 1.0, 10, 100 = Pen ch concentrations in μg). Reproduced with permission from Schwab et al., 2004.

IgE and IgG1 (Figures 6 and 7); bronchoalveolar lavage (BAL) cell counts showed increased numbers of eosinophils and neutrophils (Figure 8); and histopathological examination of mouse lungs showed perivascular inflammation by eosinophils and neutrophils as well as increased mucus production (Figure 9). These data demonstrate that sensitization to PC protease allergens released in vivo can produce a significant allergic inflammatory reaction in mice. This could have serious implications for humans as well. V PC spores can make it to the lower respiratory tract of man due to their small size. The spore, once in the human lung, can now act as a carrier and adjuvant for the Pen ch allergen. We believe that the Pen ch allergens are released by V PC spores in the lungs after they are inhaled. We have shown that PC spores remain V for up to 36 hours in the mouse lung before they are cleared by alveolar macrophages (Cooley et al., 1999). Therefore PC spores would have sufficient time to produce and release Pen ch which would then be processed by the lower respiratory tract dendritic cells (DC; Holt et al., 1999). Indeed, it has been recently shown that fungal spores are phagocytized by DC and brought to the regional lymph nodes for presentation to lymphocytes there (Bozza et al., 2002).

These studies indicate the importance of removing all PC growth sites from contaminated buildings.

Detection of airborne SC MTM on particulates smaller than conidia

Highly respirable particles with a diameter size of less than 1 micron represent a large percentage of the particles found in the IDA. It is possible, indeed likely, this particulate matter can serve as a carrier for toxic material, especially the mycotoxins produced by fungi in water- damaged buildings. As previously discussed, one of the things that has always bothered us, was the finding of people with symptoms of MTM

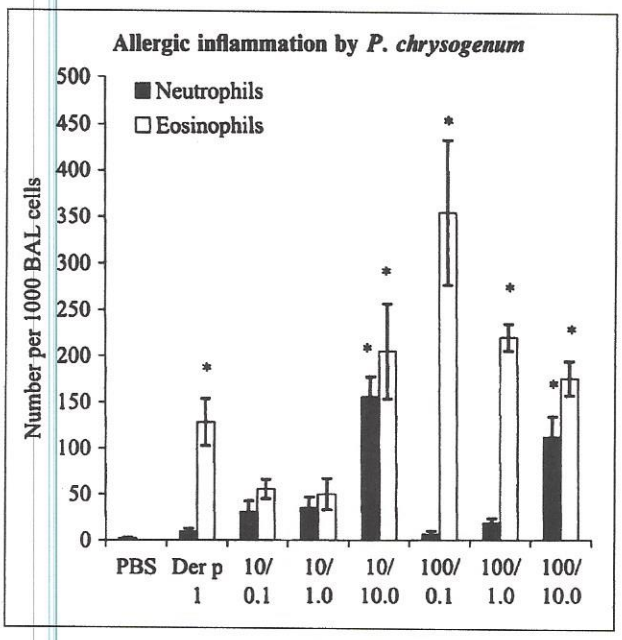

Figure 8. Airway bronchoalveolar lavage [(BAL) eosinophils and neutrophils after 7 weeks of intraperitoneal and intranasal inoculations with various concentrations of Pen ch. Bars represent the mean number of each cell type per 1000 BAL cells counted from each treatment group ($n = 6$). *$p < .02$ compared with negative control animals (phosphate-buffered saline). Error bars represent SEM (0.1, 1.0, 10, 100 = Pen ch concentrations in μg). Reproduced with permission from Schwab et al., 2004.

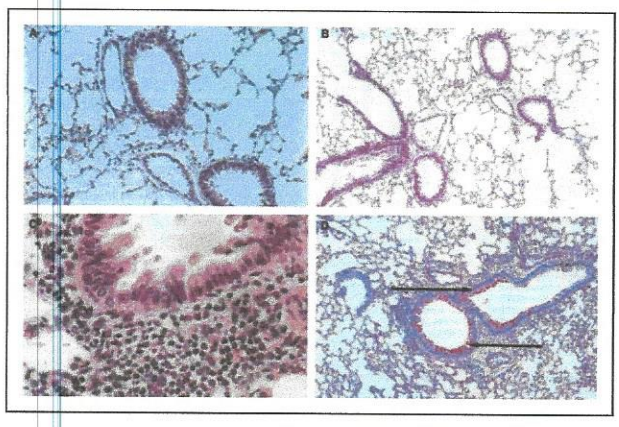

Figure 9. Histopathological examination of lungs from mice after 7 weeks of intraperitoneal and intranasal inoculation with 10 μg of Pen ch. Significant perivascular and peribronchial eosinophilia and neutrophilia are evident in mice sensitized to Pen ch (C) (H&E × 500) compared with mice treated with phosphate buffered saline (PBS) only (A), (H&E × 125). Arrows indicate increased mucus cell hyperplasis in mice sensitized to Pen ch (D) (periodic acid Schiff [PAS] × 125) compared with mice treated with PBS only (b) (PAS × 125). Reproduced with Permission from Schwab et al., 2004.

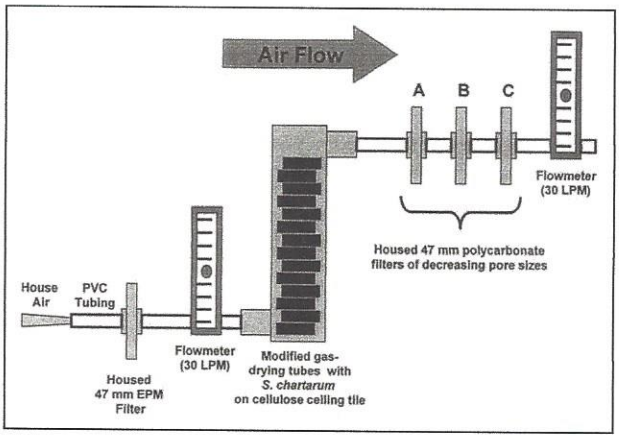

Figure 10. Experiment air sampling apparatus. Filtered house air at a flow rate of 30 L/min (LPM) was passed over cellulose ceiling tile with confluent *S. chartarum* growth for various periods of time. A total of six gas-drying tubes representing approximately 1,176 cm^2 of *S. chartarum* growth were connected using polyvinyl chloride (PVC) tubing. Particles were separated and collected on 47-mm-diameter polycarbonate membrane filters with pore sizes of 5.0 μm (filter A), 1.2 μm (filter B0 and 0.4 μm (filter C) and later were analyzed for the presence of macrocyclic trichothecenes. Reproduced with permission from Brasel et al (2005a).

exposure but with the absence of SC spores in the air in their buildings. We therefore felt that it was indeed likely that the MTM could be in the air in SC-infested buildings and not be bound to SC spores. But no one had ever been able to show that before. There were simply no techniques available that were sensitive enough to detect minute quantities of MTM. That changed in 2003 with the production of a polyclonal antibody made in rabbits against satratoxin G (SG) which is an MTM produced by SC (Chung et al., 2003). In our hands, this antibody was more sensitive than high performance liquid chromatography (HPLC) for the detection of SG, when it was used in an enzyme linked immunosorbent assay (ELISA).

Therefore, in this study (Brasel et al., 2005a), the existence of airborne SC MTM on particles smaller than spores was examined. Cellulose CT with confluent SC growth was used in gas-drying containers (GDC) through which filter air was directed. Particulates exiting the GDC were collected on a series of polycarbonate membrane filters (PMF) with decreasing pore size. A diagram of the air-sampling apparatus can be seen in Figure 10. A scanning electron microscope was used to detect SC conidia on the PMF (Figure 11). SC spores are clearly visible in Figures 11A

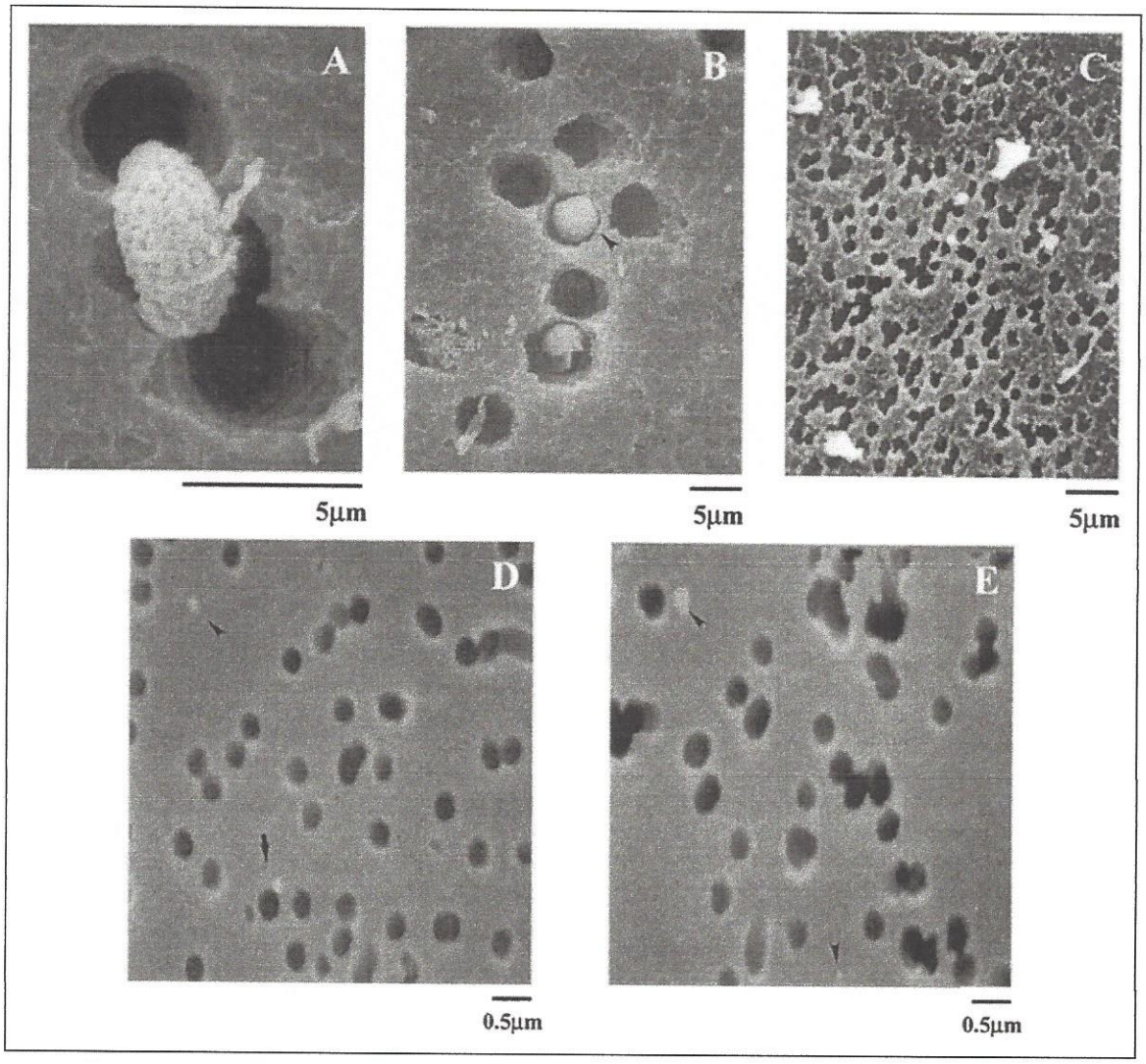

Figure 11. Scanning electron micrographs of polycarbonate membrane filters following 72 hours of sampling from the air sampling apparatus. The filter pores are clearly distinguishable from captured particulate matter (dark round and irregularly shaped bodies, respectively). (A) Filter (pore size 5.0 μm) with a captured *S. chartarum* spore. Magnification ×5000. (B) The same type of filter with an intact *S. chartarum* spore lodged in a pore (arrowhead). Magnification ×2000. (C) Filter (pore size 1.2 μm) with no *S. chartarum* spores but with a significant amount of debris. Magnification ×2500. (D and E) Filters (pore size 0.4μm) with extremely small captured particulates (arrowheads). Magnification ×10,000. Reproduced with permission from Brasel et al (2005a).

and 11B. No SC spores can be seen in Figures 11C, 11D or 11E. These figures demonstrate that the PMF do not allow SC spores to be deposited on filters of less than or equal to 1.2 micron pore size. A competitive ELISA was used to detect the presence of MTM on filter extracts. The results demonstrated that extracts from SC spore-free PMF showed statistically significant ($p < .05$) antibody binding that elevated with increased sampling time (Table 3). Cross-reactivity to various mycotoxins was investigated to examine the specificity of the anti-SG antibody. Statistically significant ($p < .05$) ELISA binding was seen for the MTM at high and low concentrations (50 ng/mL and 500 pg/mL). Of the other mycotoxins tested (Table 4), only verrucarol and diacetylverrucarol (both simple trichothecenes) showed significant binding, and then only at high concentrations (50 ng/mL). The presence of satratoxin H (SH) in the PMF spore-free extracts was suggested by HPLC (Figure 12). These data demonstrate that SC MTM can become airborne in

Table 3. Competitive enzyme-linked immunosorbent assay (ELISA) inhibition of select trichothecenes and two non-trichothecene mycotoxins

Toxin[a]	Concn[b]	% Inhibition[c]
Satratoxin G	50 ng/mL	58.4 ± 2.85[d]
	5 ng/mL	24.4 ± 1.75[d]
	500 pg/mL	9.23 ± 6.14
	50 pg/mL	0.00
Satratoxin H	50 ng/mL	79.3 ± 1.56[d]
	5 ng/mL	62.5 ± 0.62[d]
	500 pg/mL	28.2 ± 2.24[d]
	50 pg/mL	0.00[e]
Roridin A	50 ng/mL	83.5 ± 0.51
	5 ng/mL	59.8 ± 5.16
	500 pg/mL	43.3 ± 10.1
	50 pg/mL	0.00[e]
Verrucarin A	50 ng/mL	78.6 ± 1.82[d]
	5 ng/mL	51.5 ± 1.60[d]
	500 pg/mL	14.2 ± 2.22[d]
	50 pg/mL	0.00[e]
Deoxynivalenol	50 ng/mL	0.195 ± 3.33
	5 ng/mL	0.00[e]
	500 pg/mL	0.038 ± 2.59
	50 pg/mL	0.00[e]
T-2 toxin	50 ng/mL	1.19 ± 2.06
	5 ng/mL	0.00[e]
	500 pg/mL	0.00[e]
	50 pg/mL	0.00[e]
Verrucarol	50 ng/mL	18.2 ± 5.65[d]
	5 ng/mL	11.1 ± 2.95[d]
	500 pg/mL	3.40 ± 2.22
	50 pg/mL	0.00[e]
Diacetylverrucarol	50 ng/mL	51.7 ± 4.11[d]
	5 ng/mL	25.3 ± 9.20[d]
	500 pg/mL	12.1 ± 4.82[d]
	50 pg/mL	0.00[e]
Neoslaniol	50 ng/mL	5.43 ± 4.08
	5 ng/mL	6.96 ± 3.47
	500 pg/mL	20.5 ± 12.6[d]
	50 pg/mL	6.64 ± 2.72
Altenuene	50 ng/mL	0.00[e]
	5 ng/mL	0.00[e]
	500 pg/mL	0.00[e]
	50 pg/mL	0.00[e]
Sterigmatocystin	50 ng/mL	0.00[e]
	5 ng/mL	0.00[e]
	500 pg/mL	8.49 ± 1.01
	50 pg/mL	0.00[e]

[a] Satratoxins G and H, roridin A and verrucarin A are macrocyclic trichothecence mycotoxins. Deoxynivalenol, T-2 toxin, verrucarol, Diacetylverrucarol and neosolaniol are non-macrocyclic trichothecence mycotoxins. Altenuene and sterigmatocystin are non-trichothecence mycotoxins.
[b] Dilutions were made using 5% methanol in phosphate buffered saline (PBS).
[c] The results are based on solvent-only (5% methanol in PBS) controls. The values represent the results for triplicate wells. The values are averages ± standard deviations.
[d] Significant as determined by Student's *t* test ($p < .05$).
[e] Negative inhibition value converted to 0.00%. Reproduced with permission from Brasel et al (2005a).

Table 4. Competitive enzyme-linked immunosorbent assay (ELISA) inhibition for the polycarbonate filter extract-ceiling tile setup

Sampling time (h)[a]	Filer pore size (μm)[b]	% Inhibition	Avg. trichothecene equivalent (ng/mL)[c]
1	5.0	96.17 ± 0.08[d]	>500[d]
	1.2	94.82 ± 0.08[d]	335.96 ± 13.09[d]
	0.4	52.50 ± 1.00[d]	1.00 ± 0.06[d]
3	5.0	96.08 ± 0.20[d]	>500[d]
	1.2	94.57 ±0.24[d]	298.72 ± 33.95[d]
	0.4	37.24 ± 3.03	0.48 ± 0.06[d]
6	5.0	95.99 ± 0.16[d]	>500[d]
	1.2	94.88 ± 0.14[d]	348.34 ± 24.65[d]
	0.4	38.36 ± 0.32[d]	0.50 ± 0.01[d]
12	5.0	96.42 ± 0.14[d]	>500[d]
	1.2	95.45 ± 0.14[d]	473.71 ± 39.42[d]
	0.4	46.61 ± 4.19[d]	0.75 ± 0.17[d]
24	5.0	96.30 ± 0.26[d]	>500[d]
	1.2	94.50 ± 0.28[d]	289.75 ± 37.42[d]
	0.4	71.93 ± 1.18[d]	3.99 ± 0.42[d]
48	5.0	85.28 ± 0.66[d]	21.76 ± 2.62[d]
	1.2	60.16 ± 3.23[d]	1.61 ± 0.33[d]
	0.4	17.35 ± 4.90[d]	0.23 ± 0.04[d]
72	5.0	95.13 ± 0.36[d]	402.48 ± 72.68[d]
	1.2	90.71 ± 0.45[d]	73.05 ± 8.84[d]
	0.4	51.57 ± 0.75[d]	0.95 ± 0.04[d]
1	5.0[e]	15.71 ± 1.82[d]	0.22 ± 0.01[d]
	1.2[e]	11.83 ± 3.01[d]	0.20 ± 0.02[d]
	0.4[e]	3.44 ± 1.43	0.15 ± 0.01
6	5.0[e]	5.97 ± 1.61	0.17 ± 0.01
	1.2[e]	12.08 ± 0.49[d]	0.20 ± 0.00[d]
	0.4[e]	0.00[e]	0.14 ± 0.01
12	5.0[e]	32.07 ± 4.31[d]	0.39 ± 0.07[d]
	1.2[e]	9.35 ± 1.88[d]	0.18 ± 0.01[d]
	0.4[e]	18.50 ± 3.52[d]	0.24 ± 0.03[d]
24	5.0[e]	37.46 ± 2.56[d]	0.49 ± 0.05[d]
	1.2[e]	15.75 ± 6.92[d]	0.23 ± 0.05[d]
	0.4[e]	14.62 ± 1.85[d]	0.21 ± 0.01[d]
NA[g]	5.0	0.00[f]	0.13 ± 0.02
	1.2	0.00[f]	0.14 ± 0.01
	0.4	0.00[f]	0.12 ± 0.01

[a] Filters are grouped based on the order of the series and sampling time. Each sampling period experiment was performed one time.
[b] The results are based on solvent-only (5% methanol in PBS [phosphate buffered saline]) controls. The data are the results for triplicate wells. The values are averages ± standard deviation. Test groups (*Stachybotrys* on ceiling tiles) at 1, 6, 12 and 24 hours were compared to correlating sampling times using ceiling tiles along and were significantly different ($p < .05$).
[c] The values are semiquantitative and are based on the macrocyclic trichothecene standard curve. Statistical analysis was performed as described for the inhibition comparisons.
[d] Significant as determined by a one-way analysis of variance ($p < .05$).
[e] Tests performed with sterile ceiling tile alone.
[f] Negative inhibition value converted to 0.00%.
[g] NA, not applicable (tests performed with sterile filters alone). Reproduced with permission from Brasel et al (2005a).

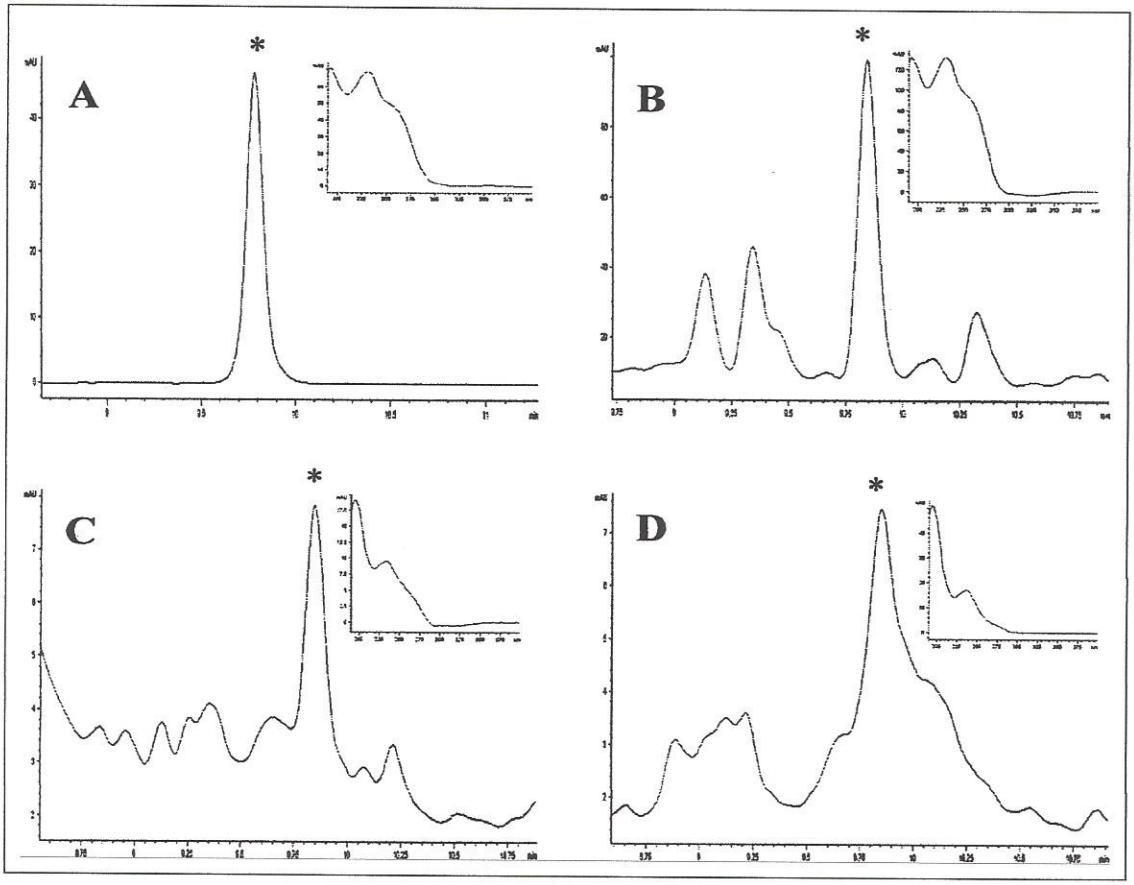

Figure 12. High performance liquid chromatography (HPLC) chromatograms of filter extracts from 120-h sampling. The retention times (minutes) are plotted on the x axis. The peak size (in milliabsorbance units) are plotted on the y axis. For UV spectrum analyses (insets), wavelengths (in nanometers) are plotted on the x axis. (A) Satratoxin H standard (indicated by an asterisk) at a concentration of 1 ng/μL, with a retention time of 9.777 min. The inset shows the UV spectrum on the toxin with a maximum absorbance near 235 nm. (B, C and D) Chromatrograms of the extracts from the 5-, 1.2- and 0.4-μm pore size filters, respectively, that were used in a 120-hour sampling experiment. The 5-μm pore size filter extract clearly shows the presence of satratoxin H with a retention time of 9.482 min (indicated by an asterisk). The results of UV spectral analyses are also present (inset). The 1.2 and 0.4-μm pore size filter extract chromatograms have major peaks at 9.845 and 9.848 min, respectively, that could be satratoxin H. UV analyses demonstrated that these two peaks were qualitatively similar to the peak for purified satratoxin H. Reproduced with permission from Brasel et al (2005a).

connection with intact SC spores or smaller particulates. This would then explain why people with symptoms of MTM intoxication are seen in SC-infected buildings where there are no SC conidia in the air.

Detection of airborne SC MTM in the indoor environment

Now that we have shown that the SC MTM were capable of separating from the SC spore in the lab, we needed to show that this phenomenon actually occurred in buildings. In order to do this, we needed a device that could concentrate large volumes of air in a very short period of time. Fortunately, such a device was made available for us to use. This machine is called the SpinCon PAS 450-10 bioaerosol sampler (BS) and can collect 450 L of air/minute (Figure 13). Therefore, armed with the ELISA that detected minute concentrations of MTM and the SpinCon PAS 450-10 BS, we were ready to investigate the presence of MTM in indoor buildings with known SC contamination (Brasel et al., 2005b). In seven mold-infested buildings, air was collected using the SpinCon PAS 450-10 BS under disturbed (fans blowing in rooms) or static conditions. An additional study was

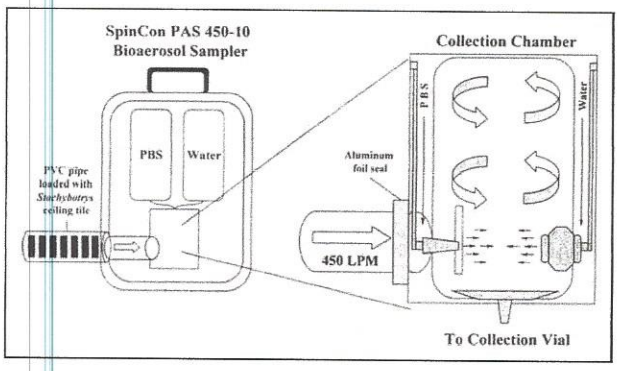

Figure 13. SpinCon PAS 450-10 bioaerosol sampler controlled setup. Polyvinyl chloride (PVC) pipe was filled with *Stachybotrys chartarum* contaminated ceiling tile and attached to the air inlet of the SpinCon sampler. Potential leaks surrounding the inlet were sealed with aluminum foil. Air passing over and through the ceiling tiles was directed into the collection chamber at a rate of 450 lpm. Aerosolized *S. chartarum* conidia and other particulate matter were captured by a swirling column of phosphate buffered saline (PBS) in the collection chamber. These trials were run in an outdoor environment. Sampling was performed for 10 and 30 min ($n = 3$ replicated for each time interval). For comparison purposes, collection was also performed using an equal area of sterile ceiling tile in the same manner. Reproduced with permission from Brasel et al., 2005b.

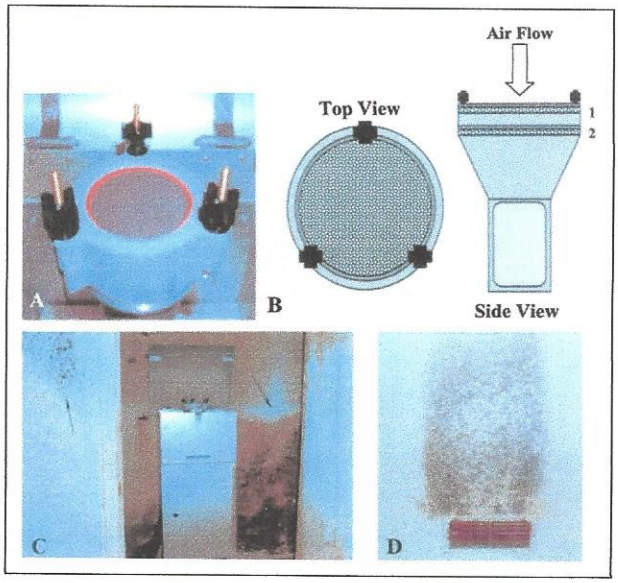

Figure 14. Andersen GPS-1 PUF high-volume air sampler set up. Panel A shows the collection module following a 24-hour sampling period. The top filter with a considerable amount of collected particulates is visible here. Panel B is a schematic of the collection module, with a top view on the left and a side view on the right. The module was modified to collect and separate particles using glass microfiber filters. Large particles, including most fungal conidia, were collected on 90-mm diamter 2.7-μm pore size GF/D filters (1), while remaining particles able to pass through the first filter were collected on highly efficient EPM filters of the same diameter (2). A heavily mold contaminated storage closet adjacent to the source of the water damage in test building 8 (shown in panel C) was chosen for sampling. Water-saturated air and ensuing fungal contamination were a result of major damage to the air conditioning unit. The degree of the damage was evident by growth near the air exit grates throughout the building (d). Reproduced with Permission from Brasel et al., 2005b.

performed using an Andersen GPS-PUF sampler that allowed for the separation and collection of particles smaller than spores (Figure 14). Four control buildings (no mold growth or water damage) and ODA were also examined. Air samples were analyzed using the SC MTM specific ELISA already described (Brasel et al., 2005a). The specificity of this ELISA was examined by using extracts of eight different fungal genera (*Aspergillus, Chaetomium, Cladosporium, Fusarium, Memnoniella, Penicillium, Rhizopus* and *Trichoderma*), five different SC strains and the IDA allergens Can f 1, Der p 1 and Fel d 1. Descriptions of the test buildings used can be seen in Table 5. Results from the test buildings showed that detectable MTM concentrations increased with sampling times and brief periods of disturbing the air with fans. MTM concentrations ranged from less than 10 to more than 1300 pg/m^3 of air (Table 6). The control buildings showed significantly lower ($p < .001$) levels of airborne MTM. ELISA specificity examination demonstrated that this assay had a very high specificity for the MTM-producing strain of SC and no other fungi. Table 7 shows the data obtained for the two buildings that were sampled using the Anderson GPS-PUF high volume sampler. This table has a column that denotes the pore size of the filter used. As expected, for each sampling time in the test building, the > 2.7 μm pore size filter demonstrated higher ELISA activity than the <3.0 μm pore size. Statistically higher MTM concentrations were seen on the filters from the environments with known SC contamination than those from the control buildings. MTM activity on filters < 0.3 μm pore size indicated mycotoxin activity on particles smaller than SC spores. These studies demonstrate that airborne MTM do exist in SC-contaminated buildings. Therefore, the presence of these compounds in the air of SC-infested buildings represents a potential health risk (Johanning et al., 1996).

Table 5. Test building descriptions

Building no.	Occupancy	Age[a] (years)	No. of rooms/ no. of samples taken	Visible water damage[b] (ft^2)	Total visible fungal contamination[c] (ft^2)	Indentified fungal genera[d]
1	Empty	10	3/11	200–300	50–100	*Stachybotrys, Chaetomium, Memnoniella, Nigrospora, Cladosporium*
2	Empty	10	2/7	>500	>500	*Stachybotrys, Aspergillus, Cladosporium, Penicillium, Alternaria, Chaetomium*
3	Occupied	10	1/4	1–10	10–20	*Chaetomium, Stachybotrys*
4	Occupied	>20	1/1	1–10	<10	*Stachybotrys*
5	Occupied	>20	2/2	50–100	50–100	*Stachybotrys, Cladosporium, Aspergillus*
6	Occupied	>20	2/4	100–200	20–50	*Chaetomium, Aspergillus*
7	Empty	>20	4/8	>500	200–300	*Memnoniella, Alternaria, Cladosporium, Aspergillus, Fusarium*
8	Empty	> 20	1/3	100–200	100–200	*Aspergillus, Alternaria, Stachybotrys, Penicillium, Fusarium, Chaetomium*

[a] Approximate age during the time of sampling.
[b] This estimate included growth that was observed following invasive inspection.
[c] Visible surface growth only. Assessment was made using the adhesive tape technique as described in the text.
[d] Fungi are listed in order of prevalence (highest to lowest). Reproduced with permission from Brasel et al., 2005b.

Table 6. Air sampling analyses of SpinCon sampled *Stachybotrys chartarum* contaminated indoor environments

Test building	Room	Dimensions of room (ft)[a]	Sampling time (min) conditions[b]	Avg % ELISA inhibition[c]	Avg. trichothecene equivalents[d] (ng/mL)	Avg. trichothecene equivalents/m^3 of sample[e] air (pg)
1	Television room	20 × 25 × 10	120, static	62.5 ± 1.9	2.0 ± 0.3	36.7 ± 4.7[f]
			120, disturbed	32.4 ± 0.5	0.42 ± 0.01	7.7 ± 0.2[f]
			30, disturbed	63.5 ± 2.9	2.2 ± 0.4	159 ± 32.1[f]
			10, disturbed	74.2 ± 1.3	5.3 ± 0.7	$1.18 \times 10^3 \pm 1.45 \times 10^2$
			120, static	21.0 ± 1.9	0.28 ± 0.02	5.2 ± 0.3
	Bedroom	20 × 25 × 8	120, static	21.0 ± 1.9	0.28 ± 0.02	5.2 ± 0.3
			120, disturbed	10.2 ± 3.5	0.20 ± 0.02	3.7 ± 0.4
			30, disturbed	18.1 ± 2.0	0.25 ± 0.02	18.8 ± 1.3[f]
			10, disturbed	9.0 ± 4.3	0.19 ± 0.02	42.9 ± 5.1
			120, static	17.2 ± 3.0	0.25 ± 0.02	4.6 ± 0.4
	Kitchen	25 × 30 × 8	120, static	17.2 ± 3.0	0.25 ±0.02	4.6 ± 0.4
			30, disturbed	17.5 ± 0.6	0.25 ± 0.00	18.3 ± 0.4[f]
			10, disturbed	30.2 ± 3.0	0.39 ± 0.04	85.9 ± 9.4
2	Kitchen	20 × 20 × 8	120, static	50.8 ± 3.5	0.98 ± 0.17	18.1 ± 3.2[f]
			120, disturbed	30.8 ± 3.6	0.40 ± 0.05	7.4 ± 1.0[f]
			30, disturbed	17.3 ± 4.0	0.25 ± 0.03	18.4 ± 2.4[f]
			120, static	16.8 ± 1.4	0.24 ± 0.01	4.5 ± 0.2
	Main entry room	20 × 25 × 8	120, static	16.8 ± 1.4	0.24 ± 0.01	4.5 ± 0.2
			120, disturbed	21.4 ± 1.4	0.28 ± 0.01	5.2 ± 0.3
			30, disturbed	49.7 ± 3.8	0.93 ± 0.20	68.8 ± 14.7[f]
			10, disturbed	50.4 ± 0.6	0.95 ± 0.03	210 ± 7.0[f]

(continued)

Table 6 (continued)

Test building	Room	Dimensions of room (ft)[a]	Sampling time (min) conditions[b]	Avg % ELISA inhibition[c]	Avg. trichothecene equivalents[d] (ng/mL)	Avg. trichothecene equivalents/m^3 of sample[e] air (pg)
3	Laundry room	6 ×8 × 8	120, static	19.0 ± 2.1	0.26 ± 0.02	4.8 ± 0.3
			10 Agg 1	95.9 ± 0.4	Above scale	Above scale[f]
			10 Agg 2	96.0 ± 0.3	Above scale	Above scale[f]
			10 Agg 3	96.1 ± 0.2	Above scale	Above scale[f]
			10 Agg 1 (1:10)	59.1 ± 3.1	1.6 ± 0.3	355 ± 65.6[f]
			10 Agg 2 (1:10)	69.5 ± 1.5	3.4 ± 0.5	757 ± 99.5[f]
			10 Agg 3 (1:10)	75.7 ± 1.0	6.2 ± 0.7	1.37 × 103 ± 1.45 × 102[f]
4	Enclosed living room area		30 Agg	63.6 ± 0.9	2.1 ± 0.5	158 ± 10.8
5	Front bathroom	10 × 8 × 8	120, static	38.5 ± 2.3	0.54 ± 0.05	10.0 ± 1.0[f]
	Back bathroom			39.4 ± 0.9	0.56 ± 0.02	10.3 ± 0.4[f]
6	Garage	15 × 25 × 8	120, static	38.3 ± 5.2	0.54 ± 0.12	10.1 ± 2.1[f]
			10, disturbed	26.1 ± 1.6	0.33 ± 0.02	73.6 ± 4.1[f]
	Kitchen	15 × 20 × 8	120, static	31.4 ± 5.3	0.41 ± 0.08	7.6 ± 1.4
			10, disturbed	24.5 ± 3.4	0.32 ± 0.04	70.1 ± 8.3
7	Hall 1	8 × 90 × 8	120, static	10.2 ± 2.4	0.20 ± 0.01	3.7 ± 0.3
			10, disturbed	7.9 ± 3.1	0.19 ± 0.02	20.7 ± 1.9
	Room 253	15 × 15 × 8	120, static	4.3 ± 2.2	0.17 ± 0.01	3.1 ± 0.2
			20, disturbed	9.6 ± 2.2	0.20 ± 0.01	21.7 ± 1.4
	Hall 2	8 × 90 × 8	120, static	4.5 ± 4.2	0.17 ± 0.02	3.2 ± 0.4
			20, disturbed	6.2 ± 0.9	0.18 ± 0.00	19.7 ± 0.5
	Room 259	15 × 15 × 8	120, static	15.3 ± 1.5	0.23 ± 0.01	4.3 ± 0.2
			20, disturbed	9.7 ± 1.6	0.20 ± 0.01	21.7 ± 1.0

ELISA, enzyme-linked immunosorbent assay.

[a] Length by width by height.

[b] Rooms were sampled under static and/or disturbed condition for noted times. Air disturbance was accomplished using 20-inch box fans on a high setting. Disturbance was allowed for 5 min prior to starting the SpinCon collection. Buildings 3 and 4 were sampled during aggressive sampling, as noted (Agg).

[c] Data are means ± standard deviations. Values are based on that for PBS alone (average of eight separately run samples). Values represent triplicate wells.

[d] Means ± standard deviations are shown. Values were derived from an ELISA-based macrocyclic tricothecene standard curve. Values represent triplicate wells.

[e] Means ± standard deviations are shown. Values represent triplicate wells. Estimated vales are based on the average trichothecene equivalents for the entire collect sample, collection time, and flow rate of the SpinCon sampler. For example, a total of 54 m^3 of air was collected for each 120-minute sample. Given a final working volume of 1 mL, trichothecene concentrations were then estimated from values obtained from ELISA testing.

[f] Values determined to be significantly different ($p < .05$) from those for control environment sampled in a similar manner. Because 20- and 30-min disturbed sampling was not performed in control environments, these samples were compared to environments sampled for 120 min under static conditions. Reproduced with permission from Brasel et al., 2005b.

However, it is still not known what levels of MTM pose a definite health risk to human beings.

Detection of trichothecene mycotoxins in sera from individuals exposed to SC in indoor environments

The last of our recent publications I would like to discuss, concerns our ability to detect MTM in human beings following exposure to SC in mold-infested buildings (Brasel et al., 2004). At the time this work was published (2004), no one had been able to demonstrate a direct human exposure to MTM in mold-contaminated buildings. Since this work was published, at least one other report has confirmed our work (Yike et al., 2006). In this study, we looked for the presence of MTM in sera from individuals exposed to indoor molds with an emphasis on SC.

Table 7. Air sampling analyses of Andersen PUF-sampled test and control indoor environments

Building and room	Dimensions of room (ft)[a]	Filer pore size (μm)	Sampling time (h)	Avg % ELISA inhibition[b]	Avg trichothecene equivalents[c] (ng/mL)	Avg trichothecene equivalents/m^3 of sample[d] air (pg)
Test building 8, garage storage closet	3 × 6 × 8	2.7	24	79.2 ± 1.6	1.5 ± 0.5	62.6 ± 12.3
		<0.3		40.1 ± 6.3	0.62 ± 0.04	6.9 ± 2.1
		2.7	48	72.0 ± 1.5	6.2 ± 0.9	14.3 ± 2.0
		<0.3		23.9 ± 3.1	0.77 ± 0.09	1.8 ± 0.2
		2.7	72	80.7 ± 0.4	16.3 ± 0.9	25.1 ± 1.4
		<0.3		52.4 ± 2.5	2.6 ± 0.4	4.1 ± 0.6
Control building 1, room 4	20 × 10 × 10	2.7	24	21.7 ± 1.5	0.41 ± 0.02	1.1 ± 0.1
		<0.3	24	0.0	0.0	0.0

ELIZA, enzyme-linked immunosorbent assay.
[a] Length by width by height
[b] Data are means ± standard deviations. Values are based on that for PBS alone (average of eight separately run samples). Values represent triplicate wells.
[c] Means ± standard deviations are shown. Values were derived from an ELISA-based macrocyclic trichothecene standard curve. Values represent triplicate wells.
[d] Means ± standard deviations are shown. Values represent triplicate wells. Estimate values are based on the average trichothecene equivalents for the entire collected sample, collection time and flow rate of the Andersen sampler. For example, a total of 216 m^3 of air was collected for each 24-hour sample. Given a final working volume of 1 mL, trichothecene concentrations were then estimated from values obtained from ELISA testing. Reproduced with permission from Brasel et al., 2005b.

Sera from people who lived or worked in mold-infested (test samples, $n = 44$) and mold-free (control samples, $n = 26$) buildings were examined using the previously described ELISA which was highly specific for MTM (Brasel et al., 2005a). Twenty-three of the test samples were significantly higher ($p < .05$) than normal human serum, whereas only one of the control samples was positive for MTM. Serum samples were divided into three distinct groups. Group 1 consisted of serum samples from individuals with documented SC exposure ($n = 18$). These individuals complained of the following symptoms: allergies, rashes, blurred vision, memory loss, headache, fatigue, nausea, balance loss, cognitive dysfunction, sinusitis, rhinitis and in some cases hemorrhage and seizures. Group 2 consisted of sera from people exposed to unidentified molds ($n = 26$). Their symptoms were congestion, numbness, cough, watery eyes, nausea, vomiting, weakness and dizziness. Group 3 consisted of sera from people with no known mold exposure ($n = 26$) and therefore represented our control group.

The average ELISA percentage inhibitions, as well as MTM concentrations for groups 1 and 2 can be seen in Tables 8 and 9, respectively. More positive sera (77.7%) can be seen in group 1 than in group 2 (34.6%). This was expected because group 1 sera came from people with known SC exposure while the sera from group 2 came from people who did not know if they had been exposed to SC. Table 10 shows the results for sera obtained for the negative controls (NC). Only one serum (NC2) showed a significant ($p < .05$) positive result in the ELISA assay. This value represented 3.8% of the NC samples. It is always possible that this individual (NC2) was exposed to SC without their knowledge. The results of this study suggest that MTM can be detected in the body tissues (in this case, serum) of people exposed to SC in mold-infested buildings. We assume that the MTM entered their bodies via inhalation, but it is possible, but less likely that they entered by absorption through the skin, or ingestion.

Recently, another group has shown the presence of MTM in human tissue following mold exposure. Yike et al. (2006), examining serum samples from three patients with documented SC exposure, revealed SG adducts to albumin in their tissue. Two of these patients were still living in their SC-infested homes whereas, one of the patients had been out of their

Table 8. Competitive enzyme-linked immunosorbent assay (ELISA) results for group 1 (documented *Stachybotrys* exposure) serum samples

Sample No.	% Inhibition[a]		Relative trichothecene concentration (ng/mL)[b]	
	Mean	SD	Mean	SD
1[c]	90.8	0.1	42.8	1.1
2[c]	92.8	0.3	83.6	9.2
3[c]	18.2	2.1	0.13	0.01
4	0.0	0.0	NA	–
5	11.1	8.9	0.11	0.03
6[c]	17.6	2.4	0.13	0.01
7	8.5	1.5	0.10	0.004
8	22.0	1.1	0.10	0.004
9	4.2	3.4	(0.09)[d]	(0.01)
10[c]	24.6	10.3	0.17[d]	0.05
11[c]	14.0	5.5	0.12[d]	0.02
12[c]	13.7	1.4	(0.07)[d]	(0.003)
13[c]	14.2	4.5	(0.07)[d]	(0.01)
14[c]	14.3	1.4	(0.07)[d]	(0.003)
15[c]	18.0	0.6	(0.08)[d]	(0.002)
16[c]	21.2	9.4	0.10	0.03
17[c]	13.3	1.6	(0.07)[d]	(0.003)
18[c]	20.9	3.5	0.15	0.02

[a] Values were based on results obtained using normal human serum when the ELISA was performed. Values represent triplicate wells.
[b] Values were obtained using the trichothecene standard curve.
[c] Significantly different from controls (normal human serum) when ELISA was performed.
[d] Values represent data that fell below the detection limit set on the standard curve. Extrapolated values are shown in parenthesis. Reproduced with permission from Brasel et al., 2004 (www.heldref.org).

Table 9. Competitive enzyme-linked immunosorbent assay (ELISA) results for group 2 (reported *Stachybotrys* exposure) serum samples

Sample No.	% Inhibition[a]		Relative trichothecene concentration (ng/mL)[b]	
	Mean	SD	Mean	SD
19	0.31	0.53	(0.06)[c]	(0.01)
20	0.00	0.0	NA	–
21	0.00	0.0	NA	–
22	0.00	0.0	NA	–
23	7.8	0.9	0.12	0.003
24	7.6	4.6	0.10	0.01
25	5.2	5.8	(0.09)[c]	(0.02)
26	3.5	3.1	(0.09)[c]	(0.01)
27	0.44	0.76	(0.08)[c]	(0.01)
28[d]	8.0	0.3	0.11	0.001
29	2.9	3.8	(0.09)[c]	(0.01)
30	4.7	4.3	0.10	0.01
31	1.5	2.1	(0.09)[c]	(0.01)
32[d]	10.0	3.4	0.12	0.01
33	3.8	1.3	0.10	0.003
34[d]	14.7	6.7	(0.08)[c]	(0.02)
35	8.8	3.9	(0.06)[c]	(0.07)
36	2.9	2.8	(0.05)[c]	(0.004)
37[d]	20.9	8.7	0.10	0.03
38[d]	21.6	9.4	0.10	0.04
39	4.0	1.8	(0.06)[c]	(0.003)
40[d]	8.8	2.6	(0.06)[c]	(0.01)
41	4.0	4.9	(0.06)[c]	(0.01)
42[d]	8.9	1.1	(0.06)[c]	(0.002)
43[d]	10.4	0.5	(0.07)[c]	(0.001)
44[d]	13.6	3.8	(0.07)[c]	(0.01)

[a] Values were based on results obtained using normal human serum when the ELISA was performed. Values represent triplicate wells.
[b] Values were obtained using the trichothecene standard curve.
[c] Values represent data that fell below the detection limit set on the standard curve. Extrapolated values are shown in parenthesis.
[d] Significantly different from controls (normal human serum) when ELISA was performed. Reproduced with permission from Brasel et al., 2004 (www.heldref.org).

home for at least 60 days. These studies indicate that we may soon be able to use SG-albumin adducts as biomarkers for SC exposure.

Future considerations and conclusions

It is now clear that the adverse health effects of the inhalation of mold and their products (e.g. conidia and mycotoxins) is a real phenomenon. In a position paper, the Centers for Disease Control (CDC) in the United States in 2002 published the following statement, 'We also know that molds can cause illness when people are exposed to extensive mold growth indoors.' In its 1993 report 'Indoor Allergens', the Institute of Medicine concluded that airborne fungal allergens were most often associated with allergic diseases, such as allergic rhinitis/conjunctivitis, allergic asthma and hypersensitivity pneumonitis (Redd, 2002). There are many publications that support the concept that the inhalation of high concentrations of fungal spores causes respiratory disease in human beings. (Blackly, 1959; Campbell et al., 1983; Dales et al., 1991; Gent et al., 2002; Jaakkola et al., 2002; Licorish et al., 1985; Straus, 2001).

The role of mycotoxins in diseases caused by fungi growing inside buildings is much more controversial. As mentioned previously, there is little doubt that as molds grow inside our buildings, they produce these toxic compounds known as mycotoxins (Croft et al., 1986; Engelhard et al., 2002; Nielsen et al., 1999; Nieminen et al., 2002; Nikulin et al., 1994; Tuomi

Table 10. Competitive enzyme-linked immunosorbent assay (ELISA) results for group 3 (Negative Controls) serum samples

Sample No. 8	% Inhibition[a]		Relative trichothecene concentration (ng/mL)[b]	
	Mean	SD	Mean	SD
NC 1	0.0	0.0	NA	
NC 2[c]	15.0	3.4	(0.08)[d]	(0.008)
NC 3	5.6	2.6	0.11	0.01
NC 4	5.4	2.2	0.11	0.01
NC 5	4.0	2.1	0.10	0.01
NC 6	9.8	3.2	(0.09)[d]	(0.01)
NC 7	2.1	2.0	0.10	0.01
NC 8	1.9	2.8	(0.05)[d]	(0.01)
NC 9	4.8	4.8	0.11	0.02
NC 10	5.3	2.0	0.11	0.01
NC 11	0.0	0.0	NA	–
NC 12	1.4	2.3	(0.08)[d]	(0.01)
NC 13	4.2	3.7	(0.09)[d]	(0.01)
NC 14	0.0	0.0	NA	–
NC 15	2.3	3.3	(0.09)[d]	(0.01)
NC 16	1.1	2.0	(0.09)[d]	(0.01)
NC 17	0.25	0.33	(0.09)[d]	(0.002)
NC 18	0.74	0.80	(0.09)[d]	(0.01)
NC 19	0.71	1.2	(0.08)[d]	(0.01)
NC 20	4.9	2.7	(0.06)[d]	(0.004)
NC 21	10.1	4.9	(0.07)[d]	(0.01)
NC 22	2.1	3.6	(0.05)[d]	(0.01)
NC 23	6.6	6.8	(0.08)[d]	(0.01)
NC 24	3.3	1.2	(0.05)[d]	(0.002)
NC 25	1.3	1.3	0.10	0.01
NC 26	5.1	4.2	(0.06)[d]	(0.01)

[a] Values were based on results obtained using normal human serum when the ELISA was performed. Values represent triplicate wells.
[b] Values were obtained using the trichothecene standard curve.
[c] Values represent data that fell below the detection limit set on the standard curve. Extrapolated values are shown in parenthesis.
[d] Significantly different from controls (normal human serum) when ELISA was performed. Reproduced with permission from Brasel et al., 2004 (www.heldref.org).

et al., 2000). The term mycotoxin literally means 'fungal toxin.' We also know that these toxins (particularly the MTM of SC) actually wind up in the body tissues of those individuals who live in SC-infested houses (Brasel et al., 2004; Yike et al., 2006). There is at least circumstantial evidence that some people have been made sick by exposure to MTM in SC-infested houses and/or buildings. For example, Croft et al. (1986) reported that a heavy SC infestation in a Chicago suburban house caused chronic health problems for the family members. The family complained of 'cold and flu symptoms, sore throats, diarrhea, headaches, fatigue, dermatitis, intermittent focal alopecia, and generalized malaise.' Highly toxic MTM were isolated from extracts of SC-contaminated household material. After the house was remediated (SC-contaminated building material removed and replaced), family members no longer complained of the above symptoms. Flappan et al. (1999) described a case of an infant with pulmonary hemorrhage (PH) where SC spores were found in the air in the patient's bedroom. In this study, it was confirmed that the SC isolate produced MTM. In this case the infant was given supportive care and survived. It was recommended by the treating physicians that the infant be housed elsewhere until the SC-contaminated house was remediated so he could safely return. In another case of a youngster with PH, physicians in Houston, Texas, USA reported the first case in which SC was isolated from the BAL fluid of a child with that disease (Elidemir et al., 1999). SC was also isolated from the child's (7 years old) water-damaged home. The child survived and completely recovered after his immediate removal from the water-damaged house. The author felt that '... this case provides further evidence that this fungus is capable of causing pulmonary hemorrhage in children.'

We do know what types of symptoms the simple trichothecenes can produce in man. This is because anguidine (a simple trichothecene produced by *Fusarium equiseti*) was injected intravenously daily into patients to examine its effectiveness as an anti-tumor drug (Goodwin et al., 1978; Murphy et al., 1978). The symptoms reported in these individuals were (among others) mental confusion, nausea, ataxia, vomiting, hypotension and drowsiness. Anguidine is also known as diacetoxyscirpenol.

Finally, the last question that remains to be answered is, do mycotoxins get into the human body via inhalations in sufficient concentrations to cause human disease? A report published in 1992 implies that they do. (Di Paolo et al., 1993). Workers in Italy reported a case of acute renal failure (ARF) caused by the inhalation of ochratoxin produced by *Aspergillus ochraceous*. A husband and wife who had spent 8 hours in a granary closed for an extended period of time experienced respiratory distress. A day later, the wife was diagnosed with ARF with above normal urine production. She also developed tubulonecrosis which resolved in 3½ weeks. While a granary would be expected to have a great deal of mold contamination, it remains to be seen whether inhalation of SC

MTM in mold-infested houses can cause the symptoms observed in cases of SBS.

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References

Blackly CH (1959) Experimental researches on the causes and nature of *catarrhus aestivus* (hay fever or hay asthma). London: Dawson Publishing Co. (reprinted from Cailliere, Tindall, and Cox, 1873), p. 57–58.

Bozza S, Gaziano R, Spreca A, et al. (2002) Dendritic cells transport conidia and hyphae of *Aspergillus fumigatus* from the airways to the draining lymph nodes and initiate disparate Th responses to the fungus. *Journal of Immunology* 168: 1362–1371.

Brasel TL, Campbell AW, Demers RE, et al. (2004) Detection of trichothecene mycotoxins in sera from individuals exposed to *Stachybotrys chartarum* in indoor environments. *Archives of Environmental Health* 59: 317–323.

Brasel TL, Douglas DR, Wilson SC, and Straus DC (2005a) Detection of airborne *Stachybotrys chartarum* macrocyclic trichothecene mycotoxins on particulates smaller than conidia. *Applied and Environmental Microbiology* 71: 114–122.

Brasel TL, Martin JM, Carriker CG, Wilson SC, and Straus DC (2005b) Detection of airborne *Stachybotrys chartarum* macrocyclic trichothecene mycotoxins in the indoor environment. *Applied and Environmental Microbiology* 71: 7376–7388.

Campbell JA, Kryda MJ, Treuhaft, MW, Marx JJ, Jr and Roberts RC (1983) Cheese worker's hypersensitivity pneumonitis. *American Reviews of Respiratory Disease* 127: 495–496.

Chung Y-J, Jarvis BB, Tak H, and Pestka JJ (2003) Immunochemical assay for satratoxin G and other macrocyclic trichothecenes associated with indoor air contamination by *Stachybotrys chartarum*. *Toxicology Mechanisms and Methods* 13: 247–252.

Cooley JD, Wong WC, Jumper CA, and Straus DC (1998) Correlation between the prevalence of certain fungi and sick building syndrome. *Occupational and Environmental Medicine* 55: 579–584.

Cooley JD, Wong WC, Jumper CJ, and Straus DC (2004) Fungi and the indoor environment: Their impact on human health. In: Straus DC (ed.) *Sick building syndrome, advances in applied microbiology*, volume 55. San Diego, CA: Elsevier Inc., p.3–30.

Cooley JD, Wong WC, Jumper CA, et al. (2000) An animal model for allergic penicilliosis induced by the intranasal instillation of viable *Penicillium chrysogenum* conidia. *Thorax* 55: 489–496.

Cooley JD, Wong WC, Jumper CA, and Straus DC (1999) Cellular and humoral responses in an animal model inhaling *Penicillium chrysogenum* spores. In: Johanning E, (ed.) *Proceedings of the third international conference on bioaerosols, fungi, and mycotoxins*, Albany, NY: Eastern New York Occupational and Environmental Health Center, p.403–410.

Croft WA, Jarvis BB, and Yatawara CS (1986) Airborne outbreak of trichothecene toxicosis. *Atmospheric Environment* 20: 549–552.

Dales RE, Burnett R, and Zwanenburg H (1991) Adverse health effects among adults exposed to home dampness and molds. *American Reviews of Respiratory Disease* 143: 505–509.

Di Paolo N, Guarnieri A, Loi F, Sacchi G, Mangiarotti AM, and Di Paolo M (1993) Acute renal failure from inhalation of mycotoxins. *Nephron* 64: 621–625.

Elidemir O, Colasurdo GN, Rossmann SN, and Fan LL (1999) Isolation of *Stachybotrys* from the lung of a child with pulmonary hemosiderosis. *Pediatrics* 104: 964–966.

Engelhart S, Loock A, Skutlarek D, et al. (2002) Occurrence of toxigenic *Aspergillus versicolor* isolates and sterigmatocystin in carpet dust from damp indoor environments. *Applied and Environmental Microbiology* 68: 3886–3890.

Flappan SM, Portnoy J, Jones P, and Barnes C (1999) Infant pulmonary hemorrhage in a suburban home with water damage and mold (*Stachybotrys atra*). *Environmental Health Perspectives* 107: 927–930.

Gent JF, Ren P, Belanger K, et al. (2002) Levels of household mold associated with respiratory symptoms in the first year of life in a cohort at risk for asthma. *Environmental Health Perspectives* 110: A781–A786.

Goodwin W, Haas CD, Fabian C, Heller-Bettinger I, and Hoogstraten B (1978) Phase 1 evaluation of anguidine (diacetoxyscirpenol, NSC-141537) *Cancer* 42: 23–26.

Gregory L, Pestka JJ, Dearborn DG, and Rand TG (2004) Localization of satratoxin-G in *Stachybotrys chartarum*

spores and spore-impacted mouse lung using immunocytochemistry. *Toxologic Pathology* 32: 26–34.

Hinkley SF, Jarvis BB (2001) Chromatographic method for *Stachybotrys* toxins. In: Trucksess MC and Pohland AE (eds.) *Methods in Molecular Biology* Vol. 157: *Mycotoxin protocols*, Totowa, NJ: Humana Press, p.173–194.

Holt PG, Stumbles PA, and McWilliam AS (1999) Functional studies on dendritic cells in the respiratory tract and related mucosal tissues. *Journal of Leukocyte Biology* 66: 272–275.

Jaakkola MS, Nordman H, Piipari R, et al. (2002) Indoor dampness and molds and development of adult-onset asthma: a population-based incident case-control study. *Environmental Health Perspectives* 110: 543–547.

Johanning E, Biagini R, Hull D, Morey P, Jarvis B, and Landsbergis P (1996) Health and immunology study following exposure to toxigenic fungi (*Stachybotrys chartarum*) in a water-damaged office environment. *International Archives of Occupational and Environmental Health* 68: 207–218.

Karunasena E, Cooley JD, Straus D, and Straus DC (2004) Protein translation inhibition by *Stachybotrys chartarum* conidia with and without the mycotoxin containing polysaccharide matrix. *Mycopathologia* 158: 87–97.

Karunasena E, Markham N, Brasel T, Cooley JD, and Straus DC (2000) Evaluation of fungal growth on cellulose containing and inorganic ceiling tile. *Mycopathologia* 150: 91–95.

Kavaler L (1965) Mushrooms, molds, and miracles: The strange realm of fungi. Colorado Springs, CO: John Day Co.

Leviticus. The Bible (Old Testament) 14:33–45.

Licorish K, Novey HS, Kozack P, Fairshter RD, and Wilson AF (1985) Role of *Alternaria* and *Penicillium* spores in the pathogenesis of asthma. *Journal of Allergy and Clinical Immunology* 76: 819–825.

McGrath JJ, Wong WC, Cooley JD, and Straus DC (1999) Continually measured fungal profiles in sick building syndrome. *Current Microbiology* 38: 33–36.

Murphy WK, Burgess MA, Valdivieso M, Livingston RB, Bodey GP, and Freireich EJ (1978) Phase 1 clinical evaluation of anguidine. *Cancer Treatment Reports* 62: 1497–1502.

Nielsen KF, Gravesen S, Nielsen PA, Andersen B, Thrane U, and Frisvad JC (1999) Production of mycotoxins on artificially and naturally infested building materials. *Mycopathologia* 145: 43–56.

Nieminen SM, Karki R, Auriola S, et al. (2002) Isolation and identification of *Aspergillus fumigatus* mycotoxins on growth medium and some building materials. *Applied and Environmental Microbiology* 68: 4871–4875.

Nikulin M, Pasanen AL, Berg S, and Hintikka EL (1994) *Stachybotrys atra* growth and toxin production in some building materials and fodder under different relative humidities. *Applied and Environmental Microbiology* 60: 3421–3429.

Redd SC. (2002) In "State of the science on molds and human health. Statement for the record before the subcommittee on oversight and investigations and housing and community opportunity on financial services", United States House of Representatives, July 18, pp 1-11.

Schwab CJ, Cooley JD, Brasel T, Jumper CA, Graham SC, and Straus DC (2003) Characterization of exposure to low levels of viable *Penicillium chrysogenum* conidia and allergic sensitization induced by a protease allergen extract from viable *P. chrysogenum* conidia in mice. *Int Arch Allergy and Immunology* 130: 200–208.

Schwab CJ, Cooley JD, Jumper CJ, Graham SC, and Straus DC (2004) Allergic inflammation induced by a *Penicillium chrysogenum* conidia-associated allergen extract in a murine model. *Allergy* 59: 758–765.

Sorenson WG, Frazer DG, Jarvis BB, Simpson J, and Robinson VA (1987) Trichothecene mycotoxins and aerosolized conidia of *Stachybotrys atra. Applied and Environmental Microbiology* 53: 1370–1375.

Straus DC (2001) Consequences of mold exposure in buildings. *Texas Journal of Rural Health* 19: 8–13.

Tuomi T, Reijula K, Johnsson T, et al. (2000) Mycotoxins in crude building materials from water-damaged buildings. *Applied and Environmental Microbiology* 66: 1899–1904.

Wilson SC, Carriker CG, Brasel TL, et al. (2004) Culturability and toxicity of sick building syndrome-related fungi over time. *Journal of Occupational and Environmental Hygiene* 1: 500–504.

Wilson SC, Straus DC (2002) The presence of fungi associated with sick building syndrome in North American zoological institutions. *Journal of Zoo and Wildlife Medicine* 33: 322–327.

Yike I, Allan T, Sorenson WG, and Dearborn DG (1999) Highly sensitive protein translation assay for trichothecene toxicity in airborne particulates: comparison with cytotoxicity assays. *Applied and Environmental Microbiology* 65: 88–94.

Yike I, Distler AM, Ziady AG, and Dearborn DG (2006) Mycotoxin adducts on human serum albumin. Biomarkers of exposure to *Stachybotrys chartarum. Environmental Health Perspectives* 114: 1221–1226.