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# The possible role of fungal contamination in sick building syndrome

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## 1. ABSTRACT

The following is a review of some of the work that we have done since 2007 regarding the importance of molds in the phenomenon of sick building syndrome (SBS). In these studies we first examined mold contamination in air handling units (AHU). Our results showed that *Cladosporium* sp. were commonly recovered in AHU as growth sites and free spores. They were found mainly on the blower wheel fan blades, the ductwork, and cooling coil fans. Our results showed that the presence of species of molds other than *Cladosporium* in locations other than the blower wheel blades indicated that the AHU condition was not optimal. The finding of *Cladosporium* species on blower fan blades does not necessarily indicate that the AHU is in poor operating condtion. In a series of three papers, we examined growth and mycotoxin production by). In these studies we showed that CG produces two potent mycotoxins, chaetoglobosin A (Ch-A) and chaetoglobosin C (Ch-C) when grown on building material. We discovered that these toxins break down when exposed to temperatures in excess of 75ºC. We also showed that growth and mycotoxin production by CG is favored at a neutral pH. In another study, we showed that mycotoxins can be detected in body fluids and human tissues from patients exposed to mycotoxin producing molds, and we showed which human tissues or fluids were the most likely to give positive results for detection of these compounds. Finally, we showed that the macrocyclic trichothecene mycotoxins (MTM) produced by *Stachybotrys chartarum* (SC) are detectable in experimental animals soon after exposure and we described the dynamics of MTM tissue loading.

## 2. INTRODUCTION

This is the third review of the work that has come out of my laboratory and my collaborator's laboratories. The previous two were a book chapter in my book in 2004 entitled, "Sick Building Syndrome" and a review I wrote in Toxicology and Industrial Health in 2009. The first review was a book chapter in "Sick Building Syndrome" entitled, "Fungi and the Indoor Environment: Their Impact on Human Health" (1). That chapter described the work that we had published on SBS from 1998 to 2002. In the second review (2), I described the work we had done from 2003 to 2006. I would like this review to be a continuation of the first two, and also to bring the reader up to date on what we know at the present time – 2010. In order to do this I would like to describe briefly what we know about SBS that I described in the two previous reviews (1, 2). First of all, we know that fungal infestation of buildings has plagued mankind since the time of Moses. This was over 3,300 years ago (3). We have come a long way in understanding why fungal infestation of buildings causes human health problems, but the most progress in this area has come in the last ten years (1, 2). We now know the identity of the important fungi that are associated with SBS, however, we probably do not know all the possible fungal compounds that are produced or their interactions with each other.

In 1998, we were the first to show a correlation between the presence of SC and *Penicillium chrysogenum* (PC) in SBS. We believed that it was the inhalation of PC spores and SC MTM from the air inside of fungally contaminated buildings that caused the human health problems so often observed in these structures (4). We then developed an animal model to attempt to determine why

the inhalation of *Penicillium* species conidia (aka spores) had the potential to cause human health difficulties (5). The results of this study showed that 42 days of inhalation of viable *Penicillium chrysogenum* spores by mice caused a type 2 T helper cell mediated inflammatory reaction.

We next studied the humoral and cellular responses of mice following the intranasal inoculation of viable PC conidia (6). These studies showed that viable PC spores were recoverable from the mouse lungs up to 1.5 days after intranasal inoculation of $10^6$ viable conidia. Because viable PC could survive inside the mouse lungs for this extended period of time, we thought they might be capable of producing immunogenic material during this time period. In the next set of experiments, we showed just that (7). In this paper, we demonstrated that mice did not develop atopic reactions to inhalational exposure to low levels ($10^2$) of viable PC. These are the levels of PC commonly found in the outside air (OSA), thus explaining why the levels of PC found in normal buildings do not lead to the symptoms commonly seen in SBS. However, the PC antigens (protease allergens) that we did isolate from viable PC did induce specific allergic reactions in the mice we examined.

We next demonstrated that when indoor air (IDA) samples are examined for their fungal organisms, they remain relatively constant (similar organisms in similar concentrations) for up to at least 6 hours (8). This means that in a fungally contaminated building, the fungal spores in the IDA do not vary dramatically over extended periods of time. In this paper, we also showed that the ODA fungal organism concentration did fluctuate wildly over the 6-hour period studies. This is not surprising because new wind currents are constantly blowing new conidia into the locale while they are continually forcing old spores away from the same area. The most important finding of this study was that "sick" buildings, once contaminated, stay that way over extended time periods. They do not get better on their own, but require remediation to provide a healthy environment again.

In the next study we attempted to determine whether or not it was feasible to develop building materials that would not permit fungal growth, even if they got wet for an extended period of time. In this study we examined the growth of PC, SC, and *Cladosporium cladosporoides* on wetted inorganic ceiling tile (ICT) and organic ceiling tile (OCT). As expected all three fungi grew on the OCT, while none of the three organisms multiplied on the ICT. This was the case even when an organic food source (tryptic soy broth) was coated on the ICT. These results showed that the production of fungi resistant building products is feasible.

We then looked at a phenomenon that had not been studied before. That was the relationship between SBS-related fungi and the difficulties encountered when trying to get animals that live in zoos to breed successfully. In our study of five zoos around the country, we found a large number of fungal species, including PC and SC, contaminating these zoos. This work demonstrated that a non-random, significant (Fisher exact test, $P<0.001$) relationship was found between proven animal morbidity and elevated levels of airborne PC (10). In a follow-up study, we examined the IDA quality of an alligator (*Alligator mississippiensis*) holding facility in the southeastern part of the United States. In this exhibit, one alligator died and all others experienced poor health. We hypothesized that the environmental conditions (microbial contamination) were associated with these issues. Nearly all surfaces we examined showed fungal growth. These were significantly higher levels of *Penicillium/Aspergillus*-like and *Chrysosporium*-like spores in the air of this facility ($P < 0.004$) when compared to the control facilities. The animals in the control facility suffered no morbidity or mortality problems. Although we were unable to demonstrate causal effects, environmental mold contamination was associated with the observed morbidity and mortality in the alligator exhibit (11). The above concludes what was discussed in the first review (1).

In the second review we attempted to answer a very perplexing question: if SC spores are not commonly found in SC contaminated structures, why do so many people who inhabit these buildings complain of health problems that could possibly be attributed to exposure to SC MTM? Our first attempt to answer this question was published in 2004 (12). In this study, we found that MTM can be displaced from the surface of an SC colony by any aqueous solution. This means that MTM can go anywhere in the building that the water goes. Once the water dries the MTM can be picked up by air currents as building dust and become airborne, where they would be easily inhaled by the building inhabitants. This theory has been put forth by other investigators (13).

We next examined the culturability and toxicity of SBS related fungi over time (14). In this study, we showed that even for extended periods of time and if no additional water was getting to a mold colony, PC and SC colonies would still be alive. We also demonstrated that the MTM produced by SC colonies remain toxic over very long periods of time (years).

In a follow-up to the 2003 study (7), we examined the allergic inflammation induced by a PC spore-associated allergen extract in a mouse model. In this study (15), CS7 black /6 mice were exposed to primary intraperitoneal (IP) injections of various amounts of a PC protease extract (Pen ch) absorbed to alum. The data obtained from this study showed that sensitization to PC protease allergens produced *in vivo* can elicit an allergic inflammatory pulmonary response in mice. It would not be unrealistic to assume that the same thing could happen in the lungs of human beings that inhaled viable PC. These studies emphasize the importance of not allowing growth sites of PC to occur in buildings occupied by people. These PC growth sites act as reservoirs of PC spores that can get into the IDA where they can be inhaled.

We next attempted to answer the question as to whether MTM could exist in the air of SC-infested buildings and not be bound to SC spores. If this was the case, then this could explain why people in infested

buildings would have symptoms of MTM exposure, but there would be no or few SC spores in the IDA (16). Therefore, we investigated the possible existence of airborne SC MTM on particles smaller than conidia. We attempted to do this employing a laboratory (17) air sampling apparatus employing SC contaminated ceiling tiles. In this study we demonstrated that SC MTM can be airborne along with intact SC conidia or smaller particles. This then explains why we see people suffering symptoms of MTM exposure where there are no or very few SC spores in the IDA (18).

Once we had shown SC MTM could separate from SC conidia in the laboratory, we attempted to demonstrate that this phenomenon could actually happen in SC infested buildings. In this study, we examined seven mold infested buildings and four control buildings with no water damage or mold growth. Air samples were analyzed employing a SpinCon PAS 450-10 bioaerosol sampler and an SC MTM specific enzyme linked immunosorbent assay (19). This study demonstrated that airborne MTM can exist in SC-infested structures thus presenting a potential health risk (19).

In the last study of the second review (2), we attempted to detect MTM in people following their exposure to SC in mold contaminated structures. In this study (16), we examined the sera from three groups of individuals. These three groups included individuals with known SC exposure (n = 18), those individuals with documented unknown mold exposure (n = 26), and a control group with no known mold exposure (n = 26). The results of this study suggested that MTM from SC can be detected in the body tissues (serum) of individuals exposed to SC in mold infested structures. It was assumed that the MTM entered their bodies via inhalation.

Other groups have confirmed these studies. Yike *et al* (20) demonstrated the presence of satratoxin G (SG) adducts to albumin in the sera of three patients with known SC exposure. One of these individuals had been out of their SC contaminated environment for at least two months, whereas the other two were still living in their SC contaminated homes when their serum was drawn. Hooper *et al*. (21) demonstrated that mycotoxins could be detected in human tissue and body fluids from patients exposed to mycotoxin producing fungi in the indoor environment. This paper will be discussed later in this review. Finally, SG, a biomarker for SC exposure was even found in the sera of cats living in a SC contaminated environment (22). These two cats suffered acute pulmonary hemorrhage during routine dental cleaning and prophylaxis. Both cats subsequently died. It was later discovered that the house was severely contaminated with mold as a result of storm damage that had occurred approximately seven months prior to the dental procedures. When the frozen sera from the two cats were examined retrospectively, it was found that SG was discovered in these body fluids. It is not surprising that domestic cats could be profoundly affected by SC MTM exposure, in this case death. These domestic animals are smaller than humans and often spend 24 hours a day indoors in the SC contaminated environment.

The following represents the most recent work done in my laboratory or the laboratories of my collaborators in the quest for a better understanding of SBS.

## 3. MOLD CONTAMINATION AND AIR HANDLING UNITS

AHU inside heating-ventilation and air conditioning systems have the potential to promote the growth of fungi and then allow for the circulation of mold spores throughout a building or a house. Therefore, it is important to know how to correctly sample an AHU. Consequently, the objectives of this study (23) were as follows: 1) To determine the mold genera and/or species most commonly found growing (or simply occurring) on selected sites in AHU. 2) To determine whether the operating condition of AHU is in any way affected by mold contamination. 3) To determine whether certain AHU areas possess mold growth sites more often than others. 4) To determine whether subsets of these AHU areas possess mold growth sites more often than others, and finally 5) to construct a microbe sampling procedure for AHU.

Four AHU sites were selected for sampling. These were based on areas where we had seen mold in the past. The locations were as follows: blower wheel, cooling coil fins, ductwork and insulation. Table 1 shows the data for all the AHU tested. A total of 566 tape lifts and 570 swab samples were collected from AHU. As can be seen from Table 1, the most common organism in mold growth sites in AHU was *Cladosporium*. The swab samples taken along with the tape lifts demonstrated that 96% of the *Cladosporium* growth sites were viable, indicating active growth sites. The swab data demonstrated that 66% of the *Penicillium* sp.isolated were viable and 100% of these were PC. Table 2 shows the incidence of the different species and types of microbes taken from the AHU surfaces. *Cladosporium cladosporiodes* was again the most common microbe found. Figure 1 shows the different fungi found as active growth sites in the various sections of the AHU. Table 3 shows the mold growth sites as determined by tape lift samples. The blower wheel fan blades contained more fungal growth sites than any of the other locations. The remaining growth site locations in orders of magnitude were insulation, ductwork, and cooling fins. This work showed that *Cladosporium* sp. were the most common organism found in AHU, and this fungus was most commonly found on the blower wheel fan blades. There was no relationship found between mold growth and the operational conditions of the AHU. It was observed however, that the AHU which contained excessive mold species in various locations did show a relatively lower operating efficiency. Indeed, mold growth sites of such fungi as *Penicillium* sp. or *Aspergillus* sp. discovered on parts of the AHU (apart from the fan blades) possibly indicates a unit in poor operating condition. It is a good idea to remove all AHU fungal growth sites. Growth sites of *Cladosporium* sp. are a common finding, most notably on blower fan wheels. A suggested sampling protocol is to take tape lifts and/or tape lift/swabs from areas of significant dirt or discoloration on the areas mentioned above, and send them to a reputable and accredited

**Table 1.** Mold growth sites and air handling unit condition rating

| | | | | Tape Lift Results | | | |
|---|---|---|---|---|---|---|---|
| School No. | AHU | Location Within AHU | No. of Samples | Fungal Group | No. of Growth Sites | Accompanying Swab Result | HVAC Score |
| 1 | 1 | Blower wheel | 12 | *Cladosporium* | 8 | *Cl. sphaerospermum* recovered from all swabs | |
| | | Insulation | -- | | | *Cl. cladosporioides* recovered from four swabs | |
| | | Coil | 15 | *Cladosporium* | 1 | *Cl. sphaerospermum* recovered from swab | |
| | | Duct | 2 | | | | 2.4 |
| | 2 | Blower Wheel | 13 | | | | |
| | | Insulation | -- | | | | |
| | | Coil | 12 | *Cladosporium* | 1 | No *Cladosporium* sp. recovered | |
| | | Duct | 2 | | | | 2.1 |
| | 3 | Blower Wheel | 13 | | | | |
| | | Insulation | -- | | | | |
| | | Coil | 12 | | | | |
| | | Duct | 2 | | | | 2.1 |
| 2 | 1 | Blower Wheel | 6 | *Cladosporium* | 1 | No *Cladosporium* sp. recovered | |
| | | Insulation | 9 | | | | |
| | | Coil | 9 | | | | |
| | | Duct | | | | | 2.7 |
| | 2 | Blower Wheel | 6 | *Aspergillus* | 1 | No *Aspergillus* sp. recovered | |
| | | Insulation | 9 | | | | |
| | | Coil | 9 | | | | |
| | | Duct | -- | | | | 2.3 |
| | 3 | Blower wheel | 6 | | | | |
| | | Insulation | 9 | *Penicillium* | 1 | *Penicillium chrysogenum* recovered from swab | |
| | | | | *Paecilomyces* | 1 | No *Paecilomyces* sp. recovered | |
| | | Coil | 9 | | | | |
| | | Duct | -- | | | | 2.7 |
| 3 | 1 | Blower wheel | 24 | *Cladosporium* | 5 | *Cl. cladosporioides* recovered from all swabs | |
| | | Insulation | -- | | | | |
| | | Coil | 26 | | | | |
| | | Duct | 8 | | | | 2.3 |
| | 2 | Blower wheel | 23 | *Cladosporium* | 10 | *Cl. cladosporioides* recovered from all swabs | |
| | | Insulation | -- | | | | |
| | | Coil | 18 | | | | |
| | | Duct | 8 | | | | 2.3 |
| 4 | 1 | Blower wheel | 6 | *Cladosporium* | 1 | *Cl. cladosporioides* recovered from swab | |
| | | Insulation | 9 | *Aspergillus* | 7 | No *Aspergillus* sp. recovered | |
| | | Coil | 9 | | | | |
| | | Duct | 9 | | | | 3.3 |
| | 2 | Blower wheel | 6 | | | | |
| | | Insulation | 9 | *Scedosporium* | 1 | No *Scedosporium* sp. recovered | |
| | | Coil | 8 | | | | |
| | | Duct | | | | | 3.4 |
| | 3 | Blower wheel | 6 | *Cladosporium* | 1 | *Cl. cladosporioides* recovered from swab | |
| | | Insulation | 9 | *Penicillium* | 1 | No *Penicillium* sp. recovered | |
| | | Coil | 9 | *Cladosporium* | 5 | *Cl. cladosporioides* recovered from two swabs | |
| | | Duct | -- | | | | 3.2 |
| 5 | 1 | Blower wheel | 8 | *Cladosporium* | 5 | *Cl. cladosporioides* recovered from all swabs | |
| | | Insulation | 9 | | | | |
| | | Coil | 9 | *Cladosporium* | 1 | *Cl. cladosporioides* recovered from swab | |
| | | Duct | -- | | | | 2.9 |
| | 2 | Blower wheel | 8 | | | | |
| | | Insulation | 9 | | | | |
| | | Coil | 9 | *Cladosporium* | 1 | *Cl. cladosporioides* recovered from swab | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | Duct | -- | | | | 3.2 |
| | 3 | Blower wheel | 3 | *Cladosporium* | 3 | *Cl. cladosporioides* recovered from all swabs | |
| | | Insulation | | | | | |
| | | Coil | 9 | | | | |
| | | Duct | | | | | 2.9 |
| | 4 | Blower wheel | 8 | *Cladosporium* | 3 | *Cl. cladosporioides* recovered from all swabs | |
| | | Insulation | 9 | | | | |
| | | Coil | 9 | | | | |
| | | Duct | | | | | 3.1 |
| 6 | 1 | Blower wheel | 7 | | | | |
| | | Insulation | -- | | | | |
| | | Coil | 9 | | | | |
| | | Duct | -- | | | | 3.9 |
| | 2 | Blower wheel | 6 | *Cladosporium* | 4 | *Cl. cladosporioides* recovered from all swabs | |
| | | | | *Aspergillus* | 1 | *Aspergillus niger* recovered from Swab | |
| | | Insulation | -- | | | | |
| | | Coil | 9 | *Cladosporium* | 1 | *Cl. cladosporioides* recovered from Swab | |
| | | | | *Acremonium* | 1 | No *Acremonium* sp. recovered | |
| | | Duct | -- | | | | 3.3 |
| | 3 | Blower wheel | 6 | *Cladosporium* | 4 | *Cl. Cladosporioides* recovered from three swabs | |
| | | | | *Penicillium* | 1 | *Penicillium chrysogenum* recovered from swab | |
| | | Insulation | -- | | | | |
| | | Coil | 9 | | | | |
| | | Duct | -- | | | | 3.1 |
| 7 | 1 | Blower wheel | 2 | *Cladosporium* | 2 | *Cl. sphaerospermum* recovered from both swabs | |
| | | Insulation | 9 | *Cladosporium* | 5 | *Cl. sphaerospermum* recovered from all swabs | |
| | | Coil | 1 | | | | |
| | | Duct | 2 | *Cladosporium* | 1 | *Cl. sphaerospermum* recovered from swab | 3.1 |
| | 2 | Blower wheel | 2 | | | | |
| | | Insulation | 8 | | | | |
| | | Coil | 1 | | | | |
| | | Duct | 1 | *Cladosporium* | 1 | *Cl. sphaerospermum* and *Cl. herbarum* recovered from each swab | 3.4 |
| | 3 | Blower wheel | 2 | | | | |
| | | Insulation | 9 | *Cladosporium* | 2 | *Cl. cladosporioides* and *Cl. sphaerospermum* | |
| | | Coil | 1 | | | recovered from each swab | |
| | | Duct | 2 | | | | 3.3 |
| | 4 | Blower wheel | 2 | | | | |
| | | Insulation | 9 | *Cladosporium* | 2 | *Cl. cladosporioides* recovered from one swab | |
| | | Coil | 1 | | | | |
| | | Duct | 2 | | | | 3.2 |
| | 5 | Blower wheel | 2 | | | | |
| | | Insulation | 9 | | | | |
| | | Coil | 1 | | | | |
| | | Duct | 2 | | | | |
| | 6 | Blower wheel | 2 | | | | |
| | | Insulation | 9 | | | | |
| | | Coil | 1 | | | | |
| | | Duct | 2 | *Cladosporium* | 1 | No *Cladosporium* sp. recovered | 3.4 |
| | 7 | Blower wheel | 2 | | | | |
| | | Insulation | 9 | | | | |
| | | Coil | 1 | | | | |
| | | Duct | 2 | | | | 3.3 |

*Note:* Scores are from a survey of 25 AHU systems from seven school buildings in Texas.

**Table 2.** Incidence of microoganisms on surfaces

| Microorganism | Percentage Recovery from 570 Swab Sampels (%) |
|---|---|
| *Cladosporium cladosporioides* | 51.23 |
| *Yeast* | 38.25 |
| *Alternaria species* | 18.77 |
| *Cladosporium sphaerospermum* | 15.26 |
| *Aspergillus niger* | 13.68 |
| *Sterile mycelia* | 12.98 |
| *No growth* | 10.70 |
| *Bacteria* | 9.65 |
| *Curvularia species* | 9.47 |
| *Nigrospora species* | 8.95 |
| *Penicillium chrysogenum* | 8.25 |
| *Chaetomium species* | 4.91 |
| *Bipolaris species* | 4.56 |
| *Aspergillus versicolor* | 4.21 |
| *Epicoccum species* | 2.98 |
| *Fusarium species* | 2.63 |
| *Penicillium citrinum* | 2.63 |
| *Chaetomium globosum* | 2.46 |
| *Trichoderma species* | 2.46 |
| *Actinomycetes* | 2.28 |
| *Engyodontium species* | 2.11 |
| *Hormographiella species* | 1.93 |
| *Cladosporium herbarum* | 1.58 |
| *Penicillium commune* | 1.58 |
| *Paecilomyces species* | 1.40 |
| *Aphanocladium species* | 1.23 |
| *Cladosporium macrocarpum* | 1.23 |
| *Paecilomyces variotii* | 1.23 |
| *Rhizopus species* | 1.05 |
| *Aspergillus flavus* | 1.05 |
| *Aspergillus sydowii* | 1.05 |
| *Aspergillus terreus* | 1.05 |
| *Penicillium aurantiogriseum* | 1.05 |
| *Aspergillus nidulans* | 0.88 |
| *Aureobasidium species* | 0.88 |
| *Penicillium oxalicum* | 0.88 |
| *Penicillium purpurogenum* | 0.88 |
| *Verticillium species* | 0.88 |
| *Aspergillus glaucus* | 0.70 |
| *Penicillium variabile* | 0.70 |
| *Penicillium funiculosum* | 0.53 |
| *Ramichloridium species* | 0.53 |
| *Acremonium species* | 0.35 |
| *Aspergillus sclerotiorum* | 0.35 |
| *Mucor species* | 0.35 |
| *Penicillium glabrum* | 0.35 |
| *Penicillium minioluteum* | 0.35 |
| *Penicillium rugulosum* | 0.35 |
| *Penicillium species* | 0.35 |
| *Ulocladium species* | 0.35 |
| *Aspergillus awamori* | 0.18 |
| *Aspergillus ustus* | 0.18 |
| *Absidia species* | 0.18 |
| *Arthrinium species* | 0.18 |
| *Aspergillus fumigatus* | 0.18 |

| | |
|---|---|
| *Aspergillus japonicus* | 0.18 |
| *Blakeslea species* | 0.18 |
| *Exophiala species* | 0.18 |
| *Ochroconis species* | 0.18 |
| *Penicillium corylophilum* | 0.18 |
| *Penicillium fellutanum* | 0.18 |
| *Penicillium miczyinskii* | 0.18 |
| *Penicillium restrictum* | 0.18 |
| *Zygosporium species* | 0.18 |

Note: A total of 570 air handling unit locations were sampled

environmental microbiology laboratory and interpret the results as described above.

## 4 . GROWTH AND MYCOTOXIN PRODUCTION BY *CHAETOMIUM GLOBOSUM*

The following paper (24) represents our attempt to understand the role of this organism in SBS. It is known that *Chaetomium* species are often encountered in buildings with indoor air quality (IAQ) problems (25, 26), but the extent of this organism's involvement in SBS is not well defined. The most common species in this genus is *C. globosum* (25). This species is the one that is most commonly isolated from water damaged structures (25, 26, 27, and 28). The organism is also known to produce two different mycotoxins called chaetoglobosins A and C (24). These mycotoxins belong to a group of compounds called cytochalasins. They act on mammalian cells by binding to actin which causes distortion in mammalian cell division. Actin produces filaments which are important in maintaining mammalian cell shape, locomotion, and cell surface projections, as well as structures in the cell (29). The purpose of this study was to 1) determine the frequency at which *Chaetomium* sp. are found in water damaged buildings (WDB) compared to other genera and 2) to examine the production *in vitro* of Ch-A and Ch-C by isolates of CG from various WDB. We first examined the frequency of isolation of *Chaetomium* from WDB with occupant complaints. Table 4 shows that *Chaetomium* species were isolated from surface samples and the air in slightly less than half of the building examined. By comparison *Alternaria*, *Aspergillus*, *Cladosporium* and *Penicillium* sp. were found far more often than *Chaetomium*, while *Paecilomyces* and *Stachybotrys* were isolated in fewer structures than *Chaetomium*. Interestingly, *Stachybotrys* and *Chaetomium* species were found less often in air samples than surface samples. This probably has something to do with the size and availability of their spores (27). We next examined the production of Ch-A and Ch-C on various agar media. Ch-A and Ch-C were detected on oatmeal agar (OA), potato dextrose agar (PDA), and malt extract agar (MEA), but not on cornmeal agar (CMA). The production of Ch-A was significantly higher on OA than PDA. The production of Ch-C was demonstrably higher on OA than the other media (Figure 2). Based on these results, we examined the production of Ch-A and Ch-C by different CG strains grown on OA. Each strain gave confluent growth on the OA plates 4 weeks post-inoculation. The number of spores produced on each plate was consistently between $10^6$ and $10^9$ conidia per isolate. Out of the different CG strains examined, 16 produced Ch-A and all CG isolates produced Ch-C (Figure 3). These data show that although *Chaetomium* sp. conidia are not isolated from air samples at a high rate, the presence of CG contamination in a WDB should not be ignored. Based on toxicity data, the inhalation of Ch-A and Ch-C has the potential to negatively affect human health. Ch-A and Ch-C have been shown to be lethal to various cell lines (31, 32). Also, injection of Ch-A was shown to be lethal when administered at relatively low doses to mice subcutaneously. The $LD_{50}$ does was determined to be 6.5 mg/kg in males and 17.8 mg/kg in female mice (30). In rats injected IP with Ch-A at doses between 2 and 16 mg/kg, all animals died within 120 minutes of exposure (32).

This study showed that *Chaetomium* species are commonly found in WDB. Also, all strains of CG we examined were capable of producing mycotoxins. We showed that chaetoglobosin production is not dependent on conidia production. Although conidia were not elaborated by CG after 4 weeks on MEA, Ch-A and Ch-C could still be detected. Therefore, these mycotoxins could be carried on fungal particulates as has been shown for SC (17). This would render these mycotoxins respirable for any persons inhabiting the WDB where CG was growing. The potential danger regarding this issue is obvious.

## 5. HEAT STABILITY OF CHAETOGLOBOSINS A AND C

We next wanted to characterize the mycotoxins Ch-A and Ch-C from CG. During the course of our purification of these two mycotoxins, we discovered that they were broken down after heating (33). We were able to show that Ch-A was significantly destroyed when exposed to various temperatures above 75º C for 24 hours, and the Ch-C preparations were also reduced in concentration (but not significantly) when exposed to the same conditions for the same length of time. When the dried methanol extracts of Ch-A and Ch-C were heated between 50 and 175˚ C for either 1 or 24 hours, after 1 hour of heating, the amounts of Ch-A and Ch-C did not significantly decrease until the temperature reached above 125˚ C (Figure 4A). The amount of Ch-A, however, decreased significantly after exposure to 100˚ (Figure 4A). Exposure to 50˚C for 1 day did not cause any loss of Ch-A or Ch-C compared to the control. A decrease in the concentration of both Ch-A and Ch-C did occur after exposure to 75˚ C for 1 day, although only Ch-A showed a significant reduction. At temperatures of 100, 125, and 150˚ C, significantly lower amounts of Ch-C were found, and no Ch-A was observed (Figure 4B).

**Table 3**. Mold growth sites as determined by tape lift sampling

| Location | No. of Locations Sampled | No. of Growth Sites | Percentage Growth Sites (%) |
|---|---|---|---|
| Blower wheels | 181 | 50 | 27.6 |
| Insulation | 143 | 19 | 13.3 |
| Duct | 35 | 5 | 14.3 |
| Fins | 207 | 14 | 6.8 |

**Table 4.** Frequency that various fungal species were isolated indoors

| Fungal genera[a] | Number of buildings[b] | Air samples[b] | Surface samples[b] |
|---|---|---|---|
| Total | 794 | 10,000 | 27,008 |
| Cladosporium | 724 (91.2%) | 7,160 (71.6%) | 2,234 (8.3%) |
| Penicillium | 731 (92.1%) | 3,374 (33.7%) | 1,546 (5.7%) |
| Alternaria | 652 (82.1%) | 2,822 (28.2%) | 531 (2.0%) |
| Aspergillus | 682 (85.9%) | 2,377 (23.8%) | 1,483 (5.5%) |
| Paecilomyces | 274 (34.5%) | 300 (3.0%) | 119 (0.4%) |
| Stachybotrys | 333 (41.9%) | 47 (0.5%) | 655 (2.4%) |
| Chaetomium | 389 (49.0%) | 89 (0.9%) | 493 (1.8%) |

a This table does not include every fungal genus that was isolated from these buildings, b The values indicate the number of buildings or samples where various fungal genera were isolated. The percentage of the total is shown in parentheses

Neither Ch-A or Ch-C were observed after samples were heated to 175˚ C for either 1 hour or 1 day (Figures 4A and 4B.)

Following that, the dried methanol extracts of Ch-A and Ch-C were heated up to 50˚ C for longer periods of time (up to 120 hours), this heating did not cause any significant loss of Ch-A or Ch-C. When the samples were heated to 50˚ C for up to 72 hours, there was no significant loss of either Ch-A or Ch-C. At 96 hours and 120 hours, the concentration of Ch-A was significantly decreased (Figure 5A). This suggested that Ch-A was not stable at this temperature for extended periods of time.

Finally, when Ch-A and Ch-C were exposed to 100˚ C or 150˚ C for 30-150 minutes or 15-75 minutes at 100˚ C, the amount of Ch-A decreased after 30 minutes. Ch-A continued to decrease with increasing time, while the concentrations of Ch-C were not significantly different from the controls (Figure 5B). After a ¼ hour exposure to 150˚ C, no Ch-A and only 50% of the concentration of Ch-C was observed when compared to controls. Also, Ch-C was observed at significantly lower levels between 30 and 75 minutes (Figure 5C). We observed that the concentrations of Ch-C increased after heating a mixture of the two mycotoxins (Figures 5A and 5B). Sebita *et al* (34) suggested that Ch-A was converted into Ch-C "by a series of keto-enol tautomerizations". It is likely that heating favors the more stable keto-form over the enol-form. This would then explain the observed increase of Ch-C over Ch-A. The data obtained in this study will aid future researchers who are attempting to elucidate the roles of Ch-A, Ch-C and CG in SBS.

## 6 . GROWTH AND MYOTOXIN PRODUCTION BY *CHAETOMIUM GLOBOSUM* IS FAVORED IN A NEUTRAL pH

This is the last paper in a series of three examining the role of CG in SBS (35). In a previous paper (24) ,we showed that the medium that demonstrated the best growth for CG also supported the highest production of Ch-A and Ch-C. Based on this study, it appears that Ch-A and Ch-C production is directly related to the growth of the organism. In this paper, we examined the influence of pH on CG growth as well as the sporulation and production of Ch-C. It is hoped that as CG growth is decreased due to sub-optimal growth conditions, the production of Ch-A and Ch-C will also decrease. This is an important consideration in SBS, because of the potential adverse health effects that Ch-A and Ch-C could cause in humans in WDB where CG is growing.

Few studies have examined the influence of pH on the growth of CG. The optimal pH range for CG growth (7.1 to 10.4) has previously been described (36). Our results indicated that CG could grow over a wide range of pHs, approximately 4.3 to 9.4. While CG grew at a pH of 3.51, the colonies that grew at a pH of 3.51 had an unusual morphology and were very small in size (Figure 6). *Chaetomium globosum* growth is best at a neutral pH (Figure 7). The effect of pH on the production of spores by CG was examined. At a pH of 4.28, 5.17, 6.07 and 7.01, perithecia were not observed one month post-inoculation. But perithecia did eventually form two months post-inoculation. After 2 months, ascospores were observed at pHs of 4.28, 5.17, and 7.01 (Table 5). Within six weeks, ascospores were produced on only Tris- buffered PDA and Tris –maleate buffered PDA. The same was true within 4 weeks for CG ascospore production (Table 5).

The formation of perithecia and ascospores by CG appears to be favored by an acidic environment. Basic conditions inhibit the formation of these structures. Therefore, building materials with a basic pH would tend to help prevent the growth of CG on wetted building materials. This would also of course limit the exposure of people in WDB to Ch-A and Ch-C.

## 7. MYCOTOXIN DETECTION IN HUMAN SAMPLES FROM PATIENTS EXPOSED TO ENVIRONMENTAL MOLDS

The objective of this study (21) was to determine if certain fungal mycotoxins could be extracted and identified in human body fluids and tissue from patients exposed to toxin producing fungi in their environment. The mycotoxins studied in this report were aflatoxins,

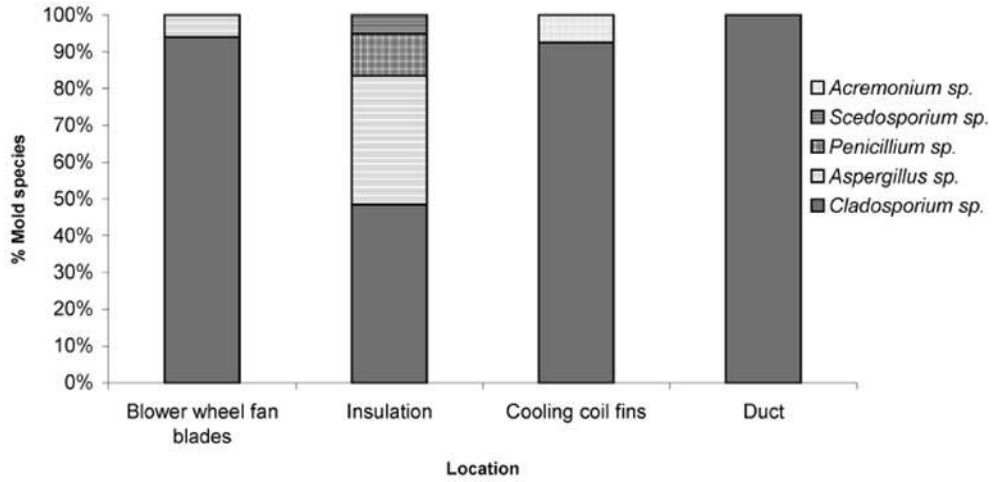


**Figure 1.** Percentages of different types of mold groups found as growth sites in different locations inside air handling units. Data were derived from 25 AHUs.

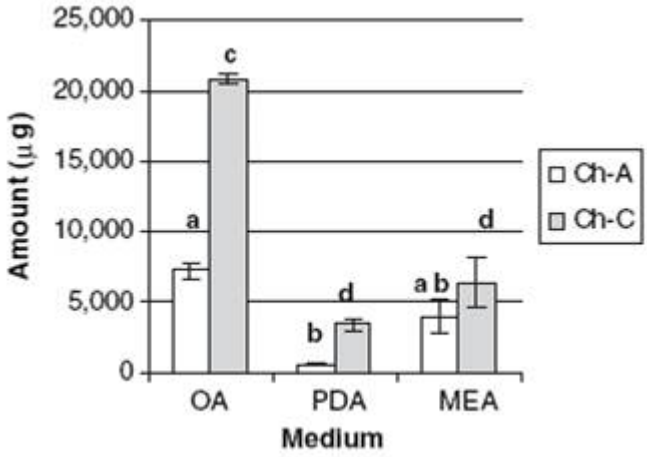


**Figure 2**. Production of chaetoglobosins A and C by C. globosum 4 weeks post-inoculation. The amount (mean ± standard error of the mean) of chaetoglobosins A and C (Ch-A and Ch-C, respectively) produced by C. globosum ATCC 16021 on five agar plates is shown (n = 9 groups with five plates per group). The following four artificial media were evaluated: potato dextrose agar (PDA), oatmeal agar (OA), cornmeal agar (CMA), and malt extract agar (MEA). The agar plates were inoculated with 500 spores of C. globosum and incubated at room temperature for 4 weeks. The presence of each toxin in the methanol extracts was detected using high performance liquid chromatography. Different letters denote a difference at the $P < 0.05$ significance level.

ochratoxins and trichothecenes. Other reports have shown that mycotoxins can be demonstrated in animal sera (22) and human sera (16, 20). Ochratoxin A has been shown to be measurable in the urine (37), while trichothecenes have also been shown to be detectable in urine (38). However, this study was the first to examine large numbers of human tissues and body fluids (obtained by the treating physician) for the presence of mycotoxins in individuals with documented exposures to toxin producing fungi in their environments.

Specimens for individuals with no known mycotoxin producing mold exposures were used as negative controls. The mycotoxin levels detectable in this group are shown in Table 6 by specimen type. In urine samples, aflatoxins levels were less than 1.0 ppb, ochratoxin levels were less than 2.0 ppb, and trichothecene levels were less that 0.2 ppb. In nasal secretion samples, aflatoxin levels were less than 1.0 ppb, ochratoxin levels were less than 2.0 ppb, and trichothecene levels were less than 0.2 ppb. In body tissues, aflatoxin levels were less than 2.0 ppb, and trichothecene levels were less than 0.2 ppb. The specificity and sensitivity of the various mycotoxin tests can be seen in Table 7. For the detection of ochratoxin in fluids and tissues, the sensitivity values ranged from 14.3 to 17.4% ($P < 0.005$). For the detection of trichothecenes in body tissue, urine and nasal secretions, the sensitivity varied from 44.4% to 94.5% ($P < 0.005$). For the detection of aflatoxins in fluids and body tissues, the sensitivity ranged from 17.4% to 70.6% ($P < 0.005$). The

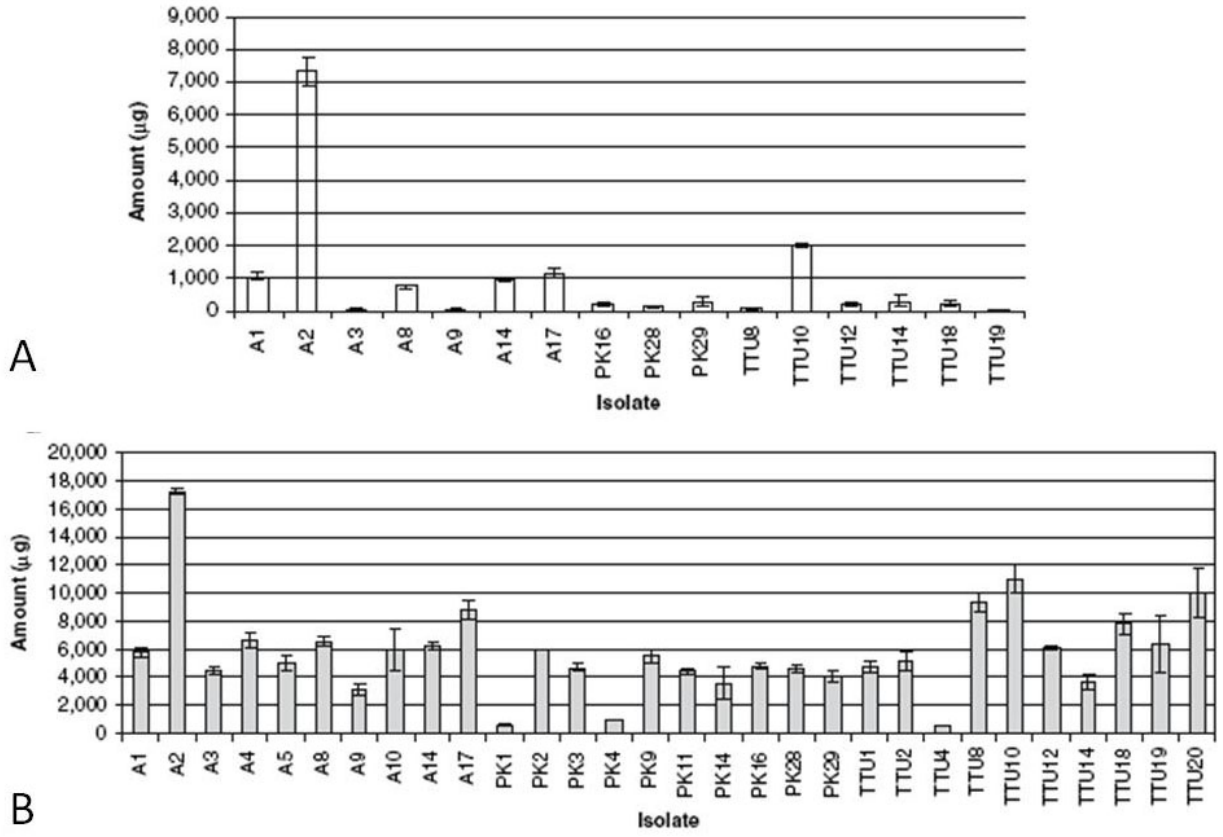


**Figure 3**. Production of chaetoglobosins A and C by different C. globosum isolates. The amount (mean ± standard error of the mean) of chaetoglobosin A (A) and chaetoglobosin C (B) produced on five oatmeal agar (OA) plates is shown (n = 3 groups with five plates per group). Prefixes indicate the laboratory where each isolate was obtained: Aerotech Laboratories (A), P&K Microbiology Services (PK) and, the Center for Indoor Air Research at Texas Tech University Health Sciences Center (TTU). Each isolate was cultured on oatmeal agar at room temperature for 4 weeks. The presence of each toxin in the methanol extracts was detected using high performance liquid chromatography

specificity was 100% in all cases. Tables 8 and 9, show the aflatoxin and ochratoxin levels in patient's tissues and body fluids, respectively. The body tissue appears to be the best specimen to test when looking for aflatoxin (Table 8). The urine appears to be the best body fluid to test when looking for ochratoxin (Table 9). Table 10 shows the trichothecene levels in patients exposed to molds and their mycotoxins. As can be seen in Table 10, the urine appears to be the best body fluid to test if one is looking for the presence of trchothecenes.

This report demonstrates that certain mycotoxins can be found in body fluids and/or human tissues of people after environmental exposure to toxin producing fungi. The discovery of mycotoxins in these tissues or fluids is consistent with the clinical symptoms reported by these individuals. Respirable MTM have been shown to be present in the air of SC-infested buildings (19. 39). The symptoms reported by the patients in this study included vomiting, aphasia, mental confusion, and nausea, as well as variations in blood pressure. The symptoms reported by these patients are similar to those reported by others in SC contaminated buildings (40). Other reports have demonstrated that SC produces MTM in WDB where the organism is actively growing, and these mycotoxins get into the air where they can be inhaled (16, 19). This report confirms that these mycotoxins can be detected in persons in amounts sufficient to potentially cause the observed health problems in people in WDB.

## 8 . DETECTION OF MACROCYCLIC TRICHOTHECENE MYCOTOXIN IN A CAPRINE (GOAT) INSTILLATION MODEL

The following study demonstrates the dynamics and detection of MTM tissue loading using a commercially available assay in a goat model (41). Previous work in our laboratory has shown the virulence of feed lot dust-associated, fungal conidia as well as the animal's response to these fungi (42). The results showed that SC was among the most virulent fungi tested in the caprine transtracheal model. Therefore, we decided to investigate in this study, the metabolism of MTMs , and their fate in goats challenged transtracheally. The first group (SC1) was challenged repeatedly with SC spores containing 1mg/kg of MTM per instillation, whereas the second group (SC2) was

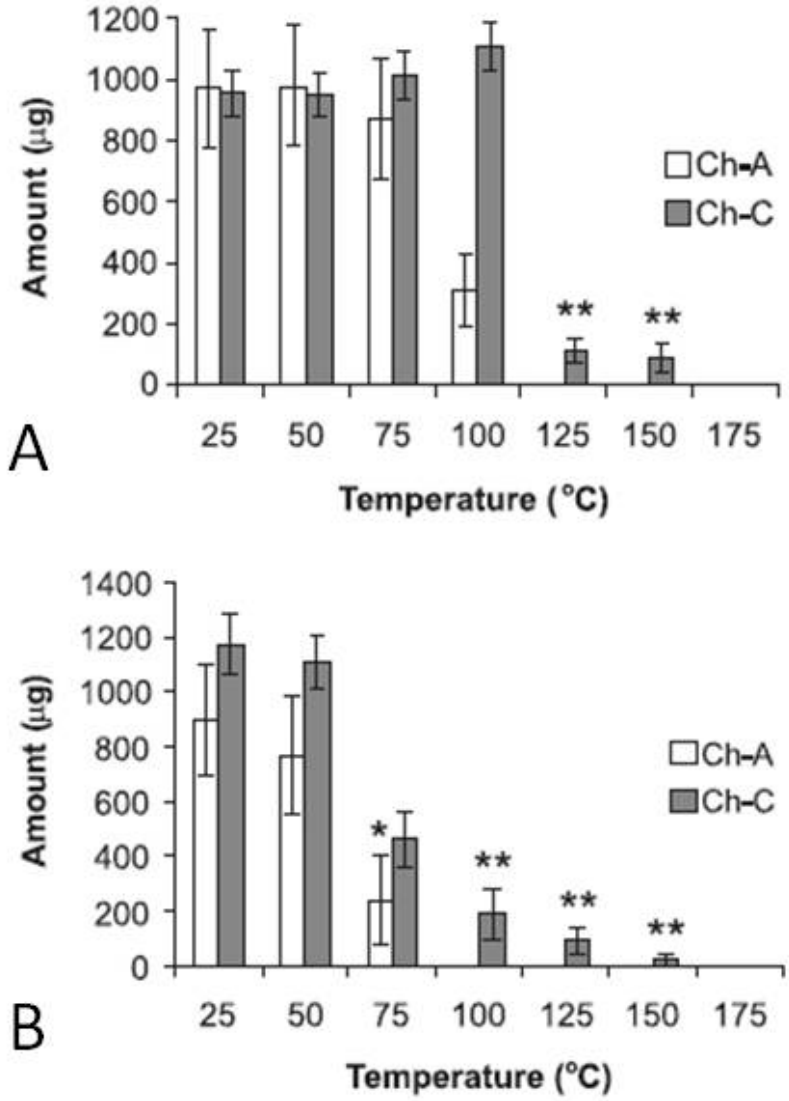


**Figure 4**. Exposure to various temperatures for 1 or 24 h. The amounts (mean and standard error of the mean) of chaetoglobosin A (Ch-A) and chaetoglobosin C (Ch-C) are shown after no treament (25ºC) or after exposure to 50, 75, 100, 125, 150, and 175ºC for either 1 h (A) or 24 h (B). Asterisks indicate a significant difference at $P < 0.05$ (single asterisk for Ch-A and double asterisks for Ch-C).

exposed one time to SC conidia with a concentration of 5µg/kg of MTM. Each spore was shown to possess 8.5 pg of MTM. Both the SC1 and SC2 groups were inoculated with SC conidial preparations and tissue and serum samples were tested from animals in these groups to determine if MTM were present, and if they were how long they remained at detectable levels. MTM was detected in the serum of three of the six animals in the SC1 group, 1 day after challenge. MTM was observed in the serum of three of the six animals from the SC1 group 1 day after challenge. One of these three animals had MTM levels at 1.63 ng/ml of serum, 72 hours after inoculation. The MTM level decreased rapidly in the serum until 120 minutes after challenge as can be seen in the SC2 group animals (Figure 8). Detectable concentrations of MTM between the 15 min. and 30 min. samples were decreased at a rate of 0.25 ng/ml. The SC1 and SC2 groups had one time point in common at 1 day after injection. The concentration of detectable MTM was similar at 1 day after challenge between the two SC groups with the mean ± standard error of the mean (SEM) of 1.69 ± 0.04 ng/ml, (SC1 group n = 3), and 2.02 ± 0.14 ng/ml for the SC2 group (n = 6). Lymph node, spleen, and lung tissues from each animal in both groups were examined for the presence of MTM. The SC1 group animals were necropsied 3 days after the last of the six challenges, and the tissues were obtained. Goats from the SC2 group were necropsied 1 day after the single instillation of SC spores and the tissues were obtained. The

**Table 5.** Effect of pH on the sporulation

| Medium | Predicted pH of buffer | Tape slide results[a] | | | | | |
|---|---|---|---|---|---|---|---|
| | | Presence of perithecia | | | Presence of ascospores | | |
| | | Week 4 | Week 6 | Week 8 | Week 4 | Week 6 | Week 8 |
| Unbuffered PDA | n/a | + | + | + | + | + | + |
| Tris buffered PDA | 7.2 | + | + | + | + | + | + |
| | 8.0 | + | + | + | + | + | + |
| | 9.0 | + | + | + | - | - | - |
| Citrate-phosphate buffered PDA | 3.0 | - | NT | NT | - | NT | NT |
| | 4.0 | - | + | + | - | - | + |
| | 5.0 | - | + | + | - | - | + |
| | 6.0 | - | - | + | - | - | - |
| | 7.0 | - | + | + | - | - | + |
| Carbonatebicarbonate buffered PDA | 9.2 | - | - | - | - | - | - |
| | 10.0 | - | - | - | - | - | - |
| | 10.7 | - | - | - | - | - | - |
| Tris-maleate buffered PDA | 5.2 | - | - | - | + | + | + |
| | 6.0 | + | + | + | + | + | + |
| | 7.0 | + | + | + | + | + | + |
| | 8.0 | + | + | + | - | + | + |
| | 8.6 | + | + | + | + | + | + |

aTape slides were taken from a single agar plate at four, six or eight weeks post-inoculation. Presenceor absence of perithecia and ascospores is indicated with a "+" or "-" respectively. Samples not takenare indicated by "NT".

**Table 6.** Mycotoxin values (in ng/mL (ppb)) by specimen in negative control group

| | | Trichothecenes | Aflatoxins | Ochratoxins |
|---|---|---|---|---|
| Specimen type | n | | | |
| Urine | 55 | < 0.2 ppb Mean: 0.08 ppb | < 1.0 ppb | < 2.0 ppb |
| Nasal Secretions | 27 | < 0.2 ppb Mean: 0.12 ppb | < 1.0 ppb | < 2.0 ppb |
| Tissue | 15 | < 0.2 ppb Mean: 0.09 ppb | < 1.0 ppb | < 2.0 ppb |

**Table 7.** Sensitivity and specificity of mycotoxin tests

| | | Sensitivity | Specificity |
|---|---|---|---|
| Trichothecenes | | | |
| | Urines | 94.5 % | 100 % |
| | Nasal Secretions | 44.4% | 100 % |
| | Tissue | 58.8 % | 100 % |
| Aflatoxins | | | |
| | Urines | 70.6 % | 100 % |
| | Nasal Secretions | 17.4 % | 100 % |
| | Tissue | 40.0 % | 100 % |
| Ochratoxins | | | |
| | Urine | 17.4 % | 100 % |
| | Nasal Secretions | ND[a] | ND |
| | Tissue | 14.3 % | 100 % |

[a]ND = not detected

**Table 8.** Specimen types tested for aflatoxins from patients exposed to molds and/or mycotoxins.

| Specimen Types | Negative Specimens | Positive specimens ≥ 1.0 ppb (ng/mL) | Number of specimens tested |
|---|---|---|---|
| Urine | 120 | 58 | 178 |
| Nasal secretions and sputa | 41 | 6 | 47 |
| Tissue block | 18* | 10** | 28 |
| Other Ear fluid (1) Spinal fluid (2) Bronchial alveolar lavage (BAL) (1) Vaginal fluid (1) | 5 | 1 (ear fluid) | 7 |
| Total | 184 | 75 | 260 |

*Brain (3), liver (4), lung (3), muscle (2), bone marrow clot (1), bladder (1), skin (3), ovary (1). ** Liver (4), brain (3), lung (2), skin (1)

**Table 9.** Specimen types tested for ochratoxin A from patients exposed to moldsand/or mycotoxins

| Specimen Types | Negative Specimens | Positive specimens ≥ 1.0 ppb (ng/mL) | Number of specimens tested |
|---|---|---|---|
| Urine | 96 | 29 | 125 |
| Nasal secretions and sputa | 26 | 1 (sputum) | 27 |
| Tissue block | 19* | 1 (renal cell carcinoma) | 20 |
| Other Ear fluid (1) Spinal fluid (2) BAL (1) | 4 | 1 | 5 |
| Total | 145 | 32 | 177 |

*Bone marrow clot (1), brain (2), liver (5), lung (5), skin (4), ovary (1), muscle (1).

**Table 10.** Specimens types tested for macrocyclic trichothecenes from patients exposed tomolds and/or mycotoxins

| Specimen Types | Negative Specimens | Positive specimens ≥ 1.0 ppb (ng/mL) | Number of specimens tested |
|---|---|---|---|
| Urine | 223 | 437 # | 660 |
| Nasal secretions (includes sputa, nasal washes, BAL) | 39 | 24 | 63 |
| Tissue | 18 * | 14 ** | 32 |
| Other | 5^ | 9^^ | 14 |
| Total | 285 | 475 | 769 |

# Sixty-two percent of all positives show 0.2 –1.0 ppb (ng/mL) in urine.* Bladder (1), ovary (1), brain (3), muscle (3), bone marrow clot (1), sinus biopsy (3), liver(3), and skin (3).** Sinus (2), lung (7), brain (2), skin (1), liver (1), ovary (1).^ Spinal fluid (2), feces (1), breast milk (1), vaginal secretions (1).^^Ear fluid (2), sputum (4), spinal fluid (1), BAL (1).

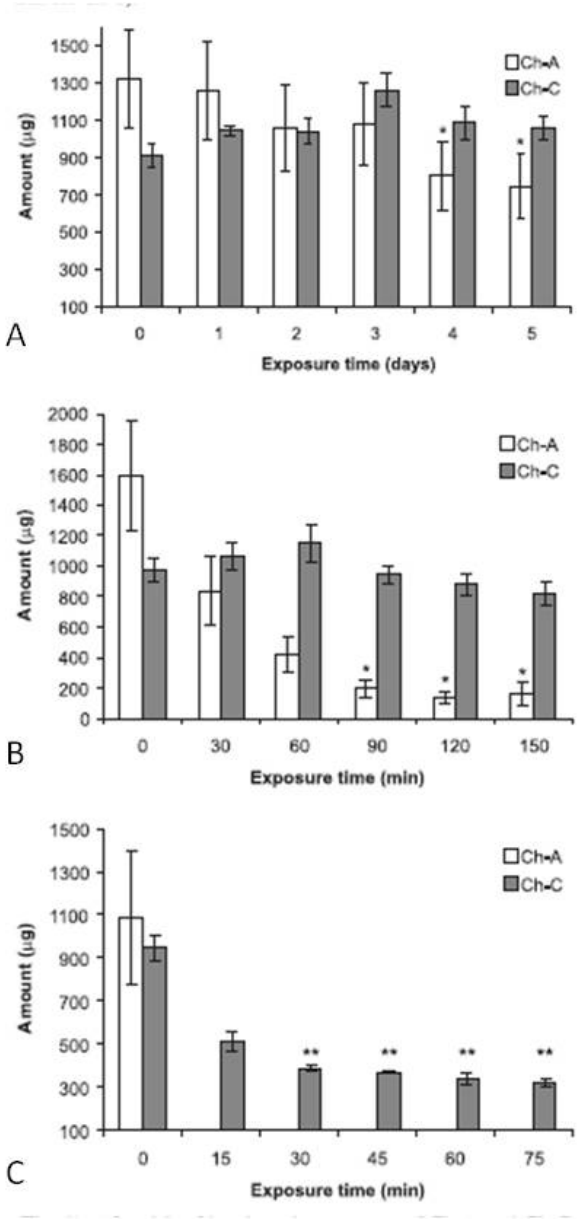


**Figure 5**. Exposure to 50, 100, or 150ºC for various times. The amounts (mean and standard error of the mean) of chaetoglobosin A (Ch-A and chaetoglobosin C (Ch-C) are shown. Control samples were left at room temperature (0 days or min). Treated samples were exposed to 50ºC over 1-5 days (A), 100ºC over 30-150 min (B), or 150ºC over 15-75 min (C). Asterisks indicate a significant difference at $P < 0.05$ (single asterisk for Ch-A and double asterisks for Ch-C).

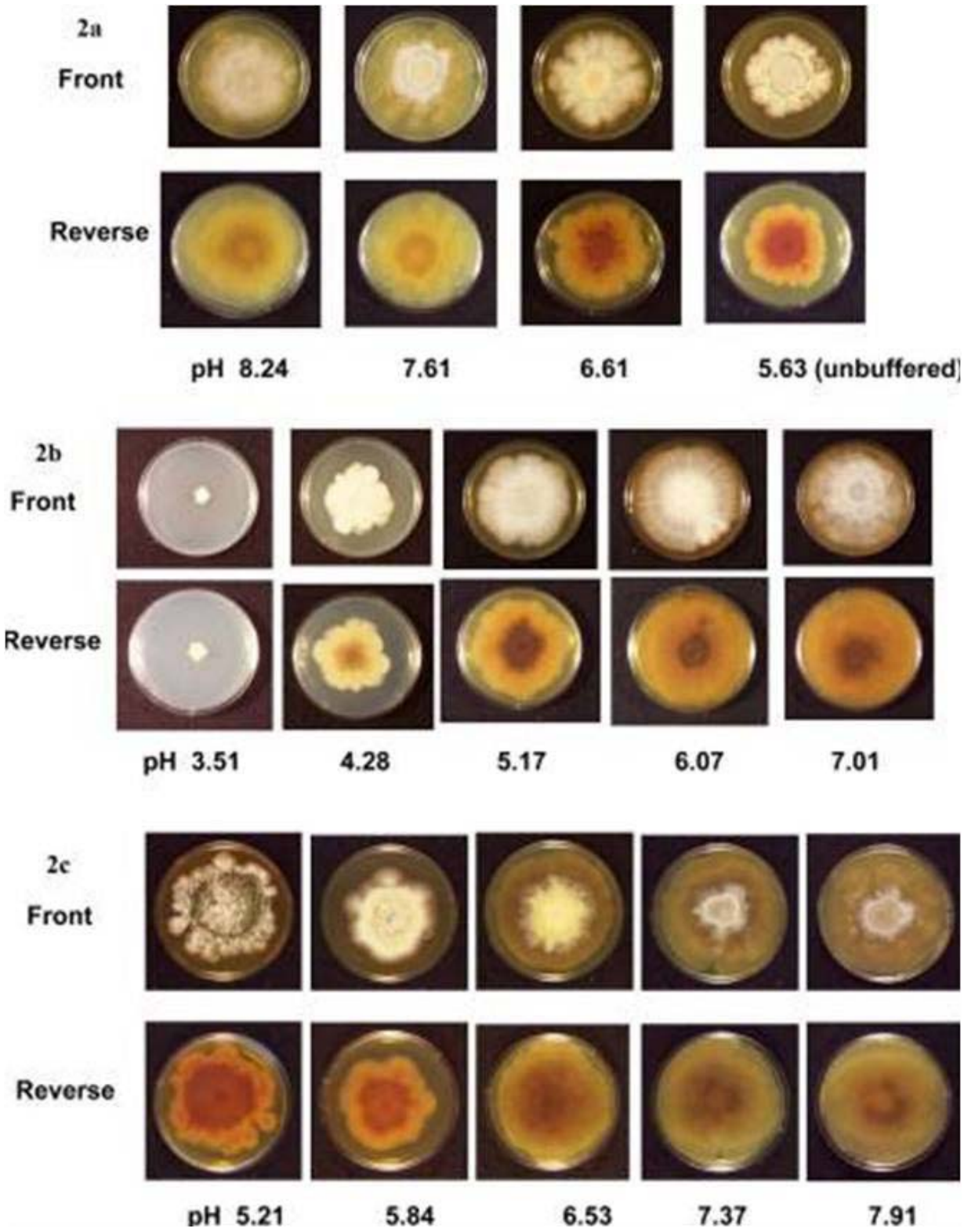


**Figure 6**. Photographs of *C. globosum* colonies at 4 weeks on Tris buffered and unbuffered potato dextrose agar (a), on citrate-phosphate buffered potato dextrose agar (b) and on Tris-maleate buffered potato dextrose agar (c). The center of each agar plate was inoculated with 500 *C. globosum* spores suspended in 20 µL of water. These photographs depict the front and reverse sides of agar plates with *C. globosum* colonies after four weeks of incubation at room temperature.

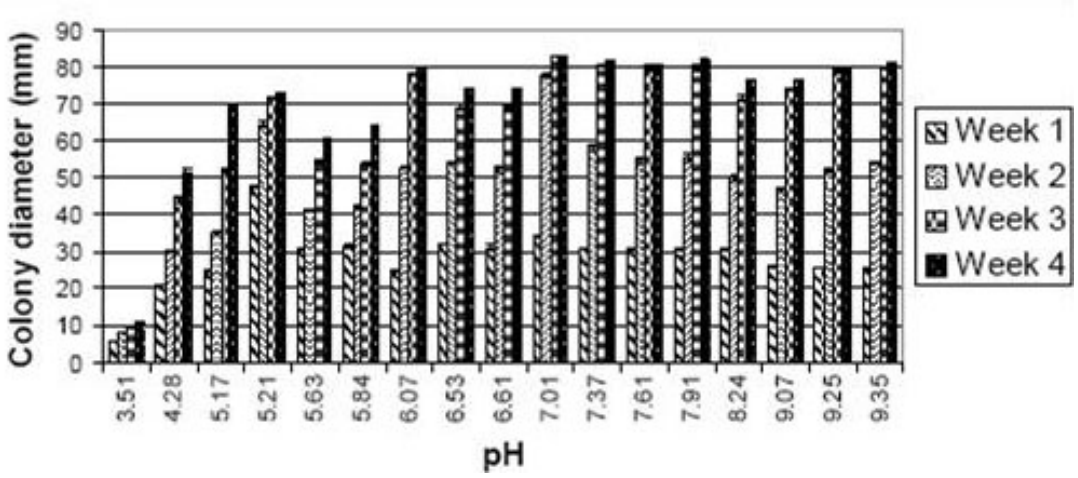


**Figure 7**. Comparison of colony diameters of *C. globosum* on buffered and unbuffered potato dextrose agar. The actual pH of each medium on the day of inoculation is listed on the x-axis. Colony diameters were measured every week. The maximum diameter of each plate was 83 mm. Mean and standard error of the mean are shown (n = 15 plates).

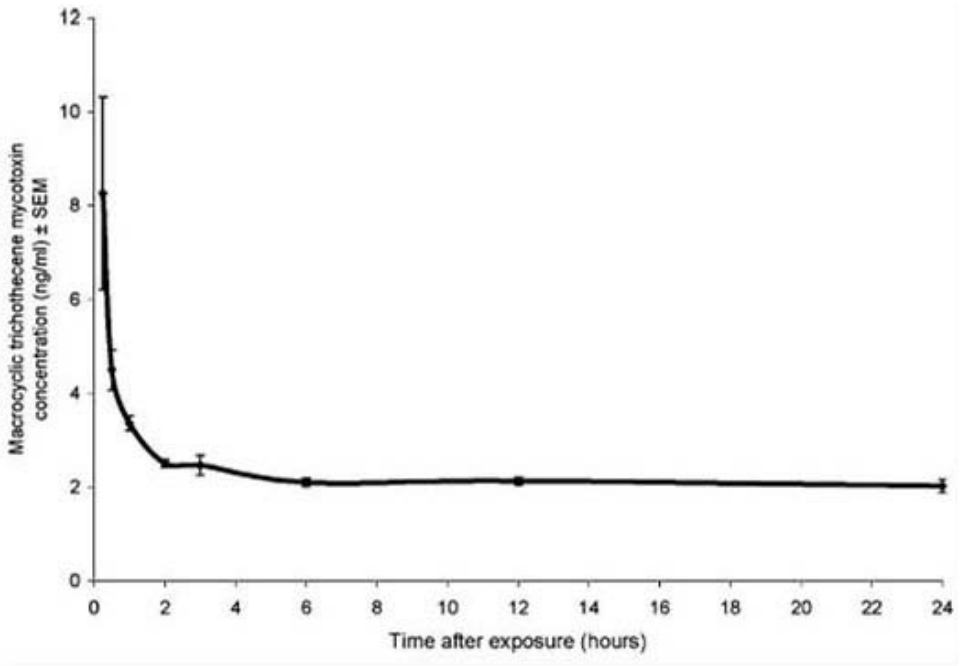


**Figure 8**. Macrocyclic trichothecene mycotoxin (MTM) concentration in serum. The curve represents the mean of all six animals from the SC2 group.

concentration of the MTM in each of the lymph node, spleen and lung from the SC1 group were 35.2, 33.7, and 34.9 ng/g, respectively (Figure 9). The concentrations of MTM in the lymph nodes, spleen, and lungs from SC2 group were 344.8; 147.0, and 158.4 ng/g, respectively.

It is important to understand how to evaluate exposure to mycotoxin producing fungi like SC. This is because this organism releases mold secondary metabolites (e.g., MTM of SC in WDB) (16, 19). However it is not known if these MTM exposures can account for any of the adverse healthy effects reported by people occupying WDB.

## 9. CONCLUSIONS

We have come a long way since the times of Moses in understanding the role of fungi in the phenomenon of SBS. We now know what fungi are involved in this phenomenon and what mold products (conidia and mycotoxins) are probably at the heart of the issue. Governmental agencies are now coming to grips within this reality. For example, in a position paper the

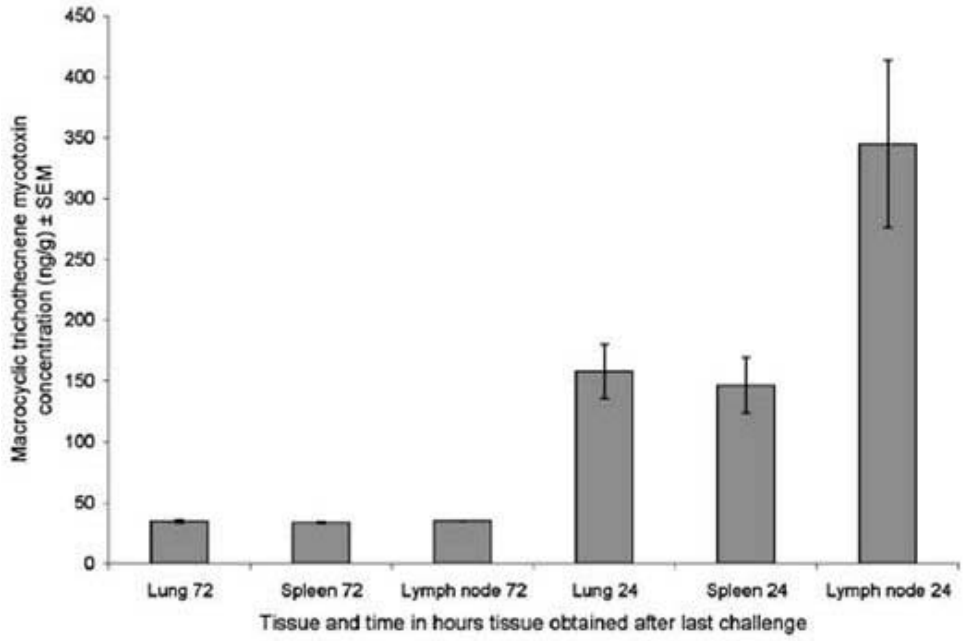


**Figure 9**. Tissue macrocyclic trichothecene mycotoxin (MTM) concentration. Tissues, as labeled, were obtained at time of necropsy. Tissues taken 72 hours after the last challenge were from the SC1 group. These animals were challenged weekly for 6 weeks at approximately 1 mg of trichothecene mycotoxin per gram of body weight. Tissues taken 24 hours after the last challenge were from the SC2 group. The values in the figure are derived from the percentage inhibition reciprocal based on tissue from the tent-control group and have been adjusted for the background.

Centers for Disease Control in the United States 2002 published the following statement, "We also know that molds can cause illness when people are exposed to extensive mold growth indoors " (44). Also, the World Health organization (WHO) made the following statement in the WHO Guidelines for Indoor Air Quality: Dampness and mould, "Sufficient epidemiological evidence is available from studies conducted in different countries and under different climatic conditions to show that the occupants of damp or moldy buildings, both houses and public buildings, are at increased risk of respiratory symptoms, respiratory infections and exacerbation of asthma. Some evidence suggests increased risk of allergic rhinitis and asthma. Although few intervention studies are available, their results show that remediation of dampness problems can reduce adverse health outcomes. There is clinical evidence that exposure to mould and other dampness related microbial agents increases the risks of rare conditions such as hypersensitivity pneumonitis, allergic alveolitis, chronic rhinosinusitis and allergic fungal sinusitis. Toxicological evidence obtained *in vivo* and *in vitro* supports these findings showing the occurrence of diverse inflammatory and toxic responses after exposure to microorganisms including their spores, metabolites and components isolated from damp buildings"(45).

In concluding this review, I would like to reiterate what we do know about SBS and what we don't. It is pretty clear that the inhalation of the high concentrations of fungal conidia (e.g., *Penicillium, Aspergillus*, *Stachybotrys*) can cause respiratory disease in man (46, 47, 48, 49, 50, 51, and 52). The role of mycotoxins in SBS is much more controversial. Nevertheless, regarding mycotoxins and their part in the causation of SBS we do know certain things. For example, we know that when fungi grow inside WDB they produce mycotoxins (53, 54, 55, 56, 57, and 58). It is known that MTM exist on the spores of SC (49, 59). We also know that the conidia of SC can be inhaled (60). We know that the MTM of SC can get into the air where they can be inhaled by inhabitants of SC-infested buildings (19). We know that the MTM are inhaled by people in WDB (16, 20, and 21). What we don't know is – do these MTM get into people in WDB in concentrations sufficient to cause the adverse health effects seen in these individuals? It is the author's hope that this final question can be answered by the next time a review is written on the recent advances in SBS research.

## 10. ACKNOWLEDGEMENTS

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